(54) Title: DNA ENCODING GALANIN GALR3 RECEPTORS AND USES THEREOF

(57) Abstract

This invention provides an isolated nucleic acid encoding a mammalian galanin receptor, an isolated galanin receptor protein, vectors comprising isolated nucleic acid encoding a mammalian galanin receptor, cells comprising such vectors, antibodies directed to a mammalian galanin receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian galanin receptor, antisense oligonucleotides complementary to unique sequences of nucleic acid encoding a mammalian galanin receptor, nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian galanin receptor, as well as methods of determining binding of compounds to mammalian galanin receptors.

DNA ENCODING GALANIN GALR3 RECEPTORS AND USES THEREOF

5   BACKGROUND OF THE INVENTION

This application is a continuation-in-part of U.S. Serial No. 08/900,230, filed July 23, 1997, which is a continuation-in-part of U.S. Serial No. 08/787,261, filed

10   January 24, 1997, which is a continuation-in-part of U.S. Serial No. 08/767,964, filed December 17, 1996, which is a continuation-in-part of U.S. Serial No. 08/728,139, filed October 9, 1996, the contents of which are incorporated by reference. Throughout this application,

15   various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for

20   these references may be found at the end of this application, preceding the sequence listing and the claims.

The neuropeptide galanin and its receptors hold great

25   promise as targets for the development of novel therapeutic agents. Galanin is widely distributed throughout the peripheral and central nervous systems and is associated with the regulation of processes such as somatosensory transmission, smooth muscle contractility,

30   hormone release, and feeding (for review, see Bartfai et al., 1993). In the periphery galanin is found in the adrenal medulla, uterus, gastrointestinal tract, dorsal root ganglia (DRG), and sympathetic neurons. Galanin released from sympathetic nerve terminals in the pancreas

35   is a potent regulator of insulin release in several species (Ahrén and Lindskog, 1992; Boyle et al., 1994), suggesting a potential role for galanin in the etiology or treatment of diabetes. High levels of galanin are observed in human and rat anterior pituitary where

40   galanin mRNA levels are potently upregulated by estrogen (Vrontakis et al., 1987; Kaplan et al., 1988). The

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released from the terminals of sensory neurons as well as spinal interneurons and appears to play a role in the regulation of pain threshold (Wiesenfeld-Hallin et al., 1992). Intrathecal galanin potentiates the anti-nociceptive effects of morphine in rats and produces analgesia when administered alone (Wiesenfeld-Hallin et al., 1993; Post et al., 1988); galanin receptor agonists may therefore be useful as analgesic agents in the spinal cord. Galanin may also play a role in the development of Alzheimer's disease. In the hippocampus galanin inhibits both the release (Fisone et al., 1987) and efficacy (Palazzi et al., 1988) of acetylcholine, causing an impairment of cognitive functions (Sundström et al., 1988). Autopsy samples from humans afflicted with Alzheimer's disease reveal a galaninergic hyperinnervation of the nucleus basalis (Chan-Palay, 1988), suggesting a role for galanin in the impaired cognition characterizing Alzheimer's disease. Together these data suggest that a galanin antagonist may be effective in ameliorating the symptoms of Alzheimer's disease (see Crawley, 1993). This hypothesis is supported by the report that intraventricular administration of the peptide galanin antagonist M35 improves cognitive performance in rats (Ögren et al., 1992). Human galanin receptors thus provide targets for therapeutic intervention in multiple CNS disorders.

High-affinity galanin binding sites have been characterized in brain, spinal cord, pancreatic islets and cell lines, and gastrointestinal smooth muscle in several mammalian species, and all show similar affinity for ^{125}I-porcine galanin (~0.5-1 nM). Nevertheless, recent *in vitro* and *in vivo* pharmacological studies in which fragments and analogues of galanin were used suggest the existence of multiple galanin receptor subtypes. For example, a galanin binding site in guinea pig stomach has been reported that exhibits high affinity for porcine

presence of galanin in the hypothalamic-pituitary-adrenal
axis coupled with its potent hormonal effects has led to
the suggestion that galanin may play an integral role in
the hormonal response to stress (Bartfai et al., 1993).

5

Within the CNS galanin-containing cell bodies are found
in the hypothalamus, hippocampus, amygdala, basal
forebrain, brainstem nuclei, and spinal cord, with
highest concentrations of galanin in the hypothalamus and
10   pituitary (Skofitsch and Jacobowitz, 1985; Bennet et al.,
1991; Merchenthaler et al., 1993). The distribution of
galanin receptors in the CNS generally complements that
of galanin peptide, with high levels of galanin binding
observed in the hypothalamus, amygdala, hippocampus,
15   brainstem and dorsal spinal cord (Skofitsch et al., 1986;
Merchenthaler et al., 1993; see Bartfai et al., 1993).
Accordingly, agents modulating the activity of galanin
receptors would have multiple potential therapeutic
applications in the CNS. One of the most important of
20   these is the regulation of food intake. Galanin injected
into the paraventricular nucleus (PVN) of the
hypothalamus stimulates feeding in satiated rats
(Kyrkouli et al., 1990), an effect which is blocked by
the peptide galanin antagonist M40 (Crawley et al.,
25   1993). In freely feeding rats, PVN injection of galanin
preferentially stimulates fat-preferring feeding (Tempel
et al., 1988); importantly, the galanin antagonist M40
administered alone decreases overall fat intake
(Leibowitz and Kim, 1992). These data indicate that
30   specific receptors in the hypothalamus mediate the
effects of galanin on feeding behavior, and further
suggest that agents acting at hypothalamic galanin
receptors may be therapeutically useful in the treatment
of human eating disorders.

35

Galanin receptors elsewhere in the CNS may also serve as
therapeutic targets. In the spinal cord galanin is

galanin (3-29) (Gu, et al. 1995), which is inactive at
CNS galanin receptors. The chimeric galanin analogue M15
(galantide) acts as antagonist at CNS galanin receptors
(Bartfai et al., 1991) but as a full agonist in
5   gastrointestinal smooth muscle (Gu et al., 1993).
Similarly, the galanin-receptor ligand M40 acts as a weak
agonist in RINm5F insulinoma cells and a full antagonist
in brain (Bartfai et al, 1993a). The pharmacological
profile of galanin receptors in RINm5F cells can be
10   further distinguished from those in brain by the
differential affinities of [D-Tyr^2]- and [D-Phe^2]-galanin
analogues (Lagny-Pourmir et al., 1989). The chimeric
galanin analogue M35 displaces ^{125}I-galanin binding to
RINm5F membranes in a biphasic manner, suggesting the
15   presence of multiple galanin receptor subtypes, in this
cell line (Gregersen et al., 1993).

Multiple galanin receptor subtypes may also co-exist
within the CNS. Galanin receptors in the dorsal
20   hippocampus exhibit high affinity for Gal (1-15) but not
for Gal (1-29) (Hedlund et al., 1992), suggesting that
endogenous proteolytic processing may release bioactive
fragments of galanin to act at distinct receptors. The
rat pituitary exhibits high-affinity binding for ^{125}I-
25   Bolton and Hunter (N-terminus)-labeled galanin (1-29) but
not for [^{125}I]Tyr^{26}-porcine galanin (Wynick et al., 1993),
suggesting that the pituitary galanin receptor is a C-
terminus-preferring subtype. Spinal cord galanin binding
sites, while similar to those in brain, show an affinity
30   for the chimeric peptide antagonist M35 intermediate
between the brain and smooth muscle (Bartfai et al.,
1991), raising the possibility of further heterogeneity.

A galanin receptor cDNA was recently isolated by
35   expression cloning from a human Bowes melanoma cell line
(Habert-Ortoli et al., 1994). The pharmacological
profile exhibited by this receptor is similar to that

observed in brain and pancreas, and on that basis the receptor has been termed GALR1. The cloned human GALR1 receptor ("hGALR1") binds native human, porcine and rat galanin with ~1 nM affinity (K_i vs. ^{125}I-galanin) and

5 porcine galanin 1-16 at a slightly lower affinity (~5nM). Porcine galanin 3-29 does not bind to the receptor. The GALR1 receptor appears to couple to inhibition of adenylate cyclase, with half-maximal inhibition of forskolin-stimulated cAMP production by 1 nM galanin, and

10 maximal inhibition occurring at about 1 μM.

Recently the rat homologue of GALR1 ("rGALR1") was cloned from the RIN14B pancreatic cell line (Burgevin, et al., (1995), Parker et al., 1995. The pharmacologic data

15 reported to date do not suggest substantial differences between the pharmacologic properties of the rat and human GALR1 receptors. Localization studies reveal GALR1 mRNA in rat hypothalamus, ventral hippocampus, brainstem, and spinal cord (Gustafson et al., 1996), regions consistent

20 with roles for galanin in feeding, cognition, and pain transmission. However, GALR1 appears to be distinct from the pituitary and hippocampal receptor subtypes described above.

25 The indication of multiple galanin receptor subtypes within the brain underscores the importance of defining galanin receptor heterogeneity at the molecular level in order to develop specific therapeutic agents for CNS disorders. Pharmacological tools capable of

30 distinguishing galanin receptor subtypes in tissue preparations are only beginning to appear. Several high-affinity peptide-based galanin antagonists have been developed and are proving useful in probing the functions of galanin receptors (see Bartfai et al., 1993), but

35 their peptide character precludes practical use as therapeutic agents. In light of galanin's multiple neuroendocrine roles, therapeutic agents targeting a

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specific disorder must be selective for the appropriate receptor subtype to minimize side effects.

Accordingly, applicants have endeavored to clone the entire family of galanin receptors for use in target-based drug design programs. The identification of non-peptide agents acting selectively only at specific galanin receptors will be greatly facilitated by the cloning, expression, and characterization of the galanin receptor family.

Applicants have recently isolated by expression cloning from a rat hypothalamic cDNA library a novel galanin receptor, termed "GALR2," not described herein, which is distinguishable from GALR1 both by its unique sequence and distinct pharmacologic properties. The GALR2 receptor is the subject of PCT International Application PCT/US97/01301, published on 31 July 1997, as WO 97/26853.

Applicants now report the isolation of a novel galanin receptor subtype, referred to herein as "GALR3," from a rat hypothalamic cDNA library. This discovery provides a novel approach, through the use of heterologous expression systems, to develop subtype selective, high-affinity non-peptide compounds that could serve as therapeutic agents for eating disorders, diabetes, pain, depression, ischemia, Alzheimer's disease, neuroendocrine disorders. The distribution of mRNA encoding the rat GALR3 receptor in multiple CNS regions as well as other organs supports the notion that the rat GALR3 is involved in these disorders. Pathophysiological disorders proposed to be linked to galanin receptor activation include eating disorders, diabetes, pain, depression, ischemia, Alzheimer's disease and reproductive disorders. Accordingly, treatment of such disorders may be effected by the administration of GALR3 receptor-selective

compounds. The presence of galanin binding sites in multiple CNS areas suggests that GALR3 receptors may also play a role in cognition, analgesia, sensory processing (olfactory, visual), processing of visceral information, motor coordination, modulation of dopaminergic activity, neuroendocrine function, sleep disorders, migraine, and anxiety.

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SUMMARY OF THE INVENTION

This invention provides an isolated nucleic acid encoding
a GALR3 galanin receptor. This invention also provides
an isolated GALR3 receptor protein. This invention also

5   provides a purified GALR3 receptor protein.   This
invention further provides DNA, cDNA, genomic DNA, RNA,
and mRNA encoding the GALR3 receptor.

This invention further provides a vector comprising the

10   GALR3 receptor.   Such a vector may be adapted for
expression of the GALR3 receptor in mammalian or non-
mammalian cells. This invention also provides a plasmid
which comprises the regulatory elements necessary for
expression of GALR3 nucleic acid in a mammalian cell

15   operatively linked to a nucleic acid encoding the GALR3
receptor so as to permit expression thereof, designated
K1086 (ATCC Accession No. 97747). This invention also
provides a plasmid which comprises the regulatory
elements necessary for expression of GALR3 nucleic acid

20   in a mammalian cell operatively linked to a nucleic acid
encoding a human GALR3 receptor so as to permit
expression thereof, designated pEXJ-hGalR3   (ATCC
Accession No. 97827). This invention provides mammalian
cells comprising the above-described plasmid or vector.

25   This invention also provides a membrane preparation
isolated from the cells.

This invention provides an isolated nucleic acid encoding
a modified GALR3 receptor, which differs from a GALR3

30   receptor by having an amino acid(s) deletion, replacement
or addition in the third intracellular domain.

This invention provides a nucleic acid probe comprising
at least 15 nucleotides, which probe specifically

35   hybridizes with a nucleic acid encoding a GALR3 receptor,
wherein the probe has a unique sequence corresponding to
a sequence present within one of the two strands of the

nucleic acid encoding the GALR3 receptor contained in
plasmid K1086. This invention still further provides a
nucleic acid probe comprising at least 15 nucleotides,
which probe specifically hybridizes with a nucleic acid

5   encoding a GALR3 receptor, wherein the probe has a unique
sequence corresponding to a sequence present within (a)
the nucleic acid sequence described in Figure 1 (Seq. ID
No. 1) or (b) the reverse complement to the nucleic acid
sequence shown in Figure 1 (Seq. ID No. 1).

10
In yet another embodiment, the GALR3 receptor is the rat
GALR3 receptor having substantially the same amino acid
sequence as the amino acid sequence shown in Figure 2.
In another embodiment, the GALR3 receptor is the rat

15   GALR3 receptor having the amino acid sequence shown in
Figure 2. In another embodiment, the GALR3 receptor is
the human GALR3 receptor. In another embodiment, the
GALR3 receptor is the human GALR3 receptor encoded by the
coding sequence of plasmid pEXJ-hGalR3. This invention

20   also provides a nucleic acid probe comprising at least 15
nucleotides, which probe specifically hybridizes with a
nucleic acid encoding a GALR3 receptor, wherein the probe
has a unique sequence corresponding to a sequence present
within one of the two strands of the nucleic acid

25   encoding the GALR3 receptor contained in plasmid pEXJ-
hGalR3. This invention provides a nucleic acid probe
comprising at least 15 nucleotides, which probe
specifically hybridizes with a nucleic acid encoding a
GALR3 receptor, wherein the probe has a unique sequence

30   corresponding to a sequence present within (a) the
nucleic acid sequence described in Figure 3 (Seq. ID No.
3) or (b) the reverse complement to the nucleic acid
sequence shown in Figure 3 (Seq. ID No. 3).

35   This invention further provides a nucleic acid probe
comprising a nucleic acid molecule of at least 15
nucleotides which is complementary to a unique fragment

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of the sequence of a nucleic acid molecule encoding a GALR3 receptor.

5   This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to the antisense sequence of a unique fragment of the sequence of a nucleic acid molecule encoding a GALR3 receptor.

10   This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a GALR3 galanin receptor, so as to prevent translation of the mRNA. This invention also provides an antisense oligonucleotide having a sequence capable of
15   specifically hybridizing to the genomic DNA molecule encoding a GALR3 receptor.

This invention provides an antibody directed to a GALR3 receptor. This invention also provides a monoclonal
20   antibody directed to an epitope of a GALR3 receptor, which epitope is present on the surface of a cell expressing a GALR3 receptor.

This invention provides a pharmaceutical composition
25   comprising an amount of the oligonucleotide effective to reduce activity of a GALR3 receptor by passing through a cell membrane and binding specifically with mRNA encoding a GALR3 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier
30   capable of passing through a cell membrane. In an embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme.

35   This invention provides a pharmaceutical composition comprising an amount of an antagonist effective to reduce the activity of a GALR3 receptor and a pharmaceutically

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acceptable carrier.

This invention provides a pharmaceutical composition comprising an amount of an agonist effective to increase activity of a GALR3 receptor and a pharmaceutically acceptable carrier.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a GALR3 receptor. This invention provides a transgenic nonhuman mammal comprising a homologous recombination knockout of the native GALR3 receptor. This invention provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a GALR3 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GALR3 receptor and which hybridizes to mRNA encoding a GALR3 receptor thereby reducing its translation.

This invention also provides a process for determining whether a compound can specifically bind to a GALR3 receptor which comprises contacting a cell transfected with and expressing DNA encoding the GALR3 receptor with the compound under conditions permitting binding of compounds to such receptor, and detecting the presence of any such compound specifically bound to the GALR3 receptor, so as to thereby determine whether the ligand specifically binds to the GALR3 receptor.

This invention provides a process for determining whether a compound can specifically bind to a GALR3 receptor which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR3 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the compound under conditions permitting binding of compounds to such receptor, and detecting the presence of the

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compound specifically bound to the GALR3 receptor, so as to thereby determine whether the compound specifically binds to the GALR3 receptor.

5   In one embodiment, the GALR3 receptor is a mammalian GALR3 receptor. In another embodiment, the GALR3 receptor is a rat GALR3 receptor. In still another embodiment, the GALR3 receptor has substantially the same amino acid sequence encoded by the plasmid K1086. In a
10   still further embodiment, the GALR3 receptor has the amino acid sequence encoded by the plasmid K1086. In another embodiment, the GALR3 receptor is a human GALR3 receptor.

15   This invention provides a process for determining whether a compound is a GALR3 receptor agonist which comprises contacting a cell transfected with and expressing DNA encoding the GALR3 receptor with the compound under conditions permitting the activation of the GALR3
20   receptor, and detecting an increase in GALR3 receptor activity, so as to thereby determine whether the compound is a GALR3 receptor agonist.

This invention provides a process for determining whether
25   a compound is a GALR3 receptor antagonist which comprises contacting a cell transfected with and expressing DNA encoding the GALR3 receptor with the compound in the presence of a known GALR3 receptor agonist, such as galanin, under conditions permitting the activation of
30   the GALR3 receptor, and detecting a decrease in GALR3 receptor activity, so as to thereby determine whether the compound is a GALR3 receptor antagonist.

This invention provides a compound determined by the
35   above-described processes. In one embodiment of the above-described processes, the compound is not previously known. In another embodiment, the compound is not known

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to bind a GALR3 receptor.

This invention provides a method of screening a plurality
of chemical compounds not known to bind to a GALR3
5   receptor to identify a compound which specifically binds
to the GALR3 receptor, which comprises (a) contacting
cells transfected with and expressing DNA encoding the
GALR3 receptor with a compound known to bind specifically
to the GALR3 receptor; (b) contacting the preparation of
10   step (a) with the plurality of compounds not known to
bind specifically to the GALR3 receptor, under conditions
permitting binding of compounds known to bind the GALR3
receptor; (c) determining whether the binding of the
compound known to bind to the GALR3 receptor is reduced
15   in the presence of the compounds, relative to the binding
of the compound in the absence of the plurality of
compounds; and if so (d) separately determining the
binding to the GALR3 receptor of each compound included
in the plurality of compounds, so as to thereby identify
20   the compound which specifically binds to the GALR3
receptor.

This invention provides a method of screening a plurality
of chemical compounds not known to activate a GALR3
25   receptor to identify a compound which activates the GALR3
receptor which comprises (a) contacting cells transfected
with and expressing the GALR3 receptor with the plurality
of compounds not known to activate the GALR3 receptor,
under conditions permitting activation of the GALR3
30   receptor; (b) determining whether the activity of the
GALR3 receptor is increased in the presence of the
compounds; and if so (c) separately determining whether
the activation of the GALR3 receptor is increased by each
compound included in the plurality of compounds, so as to
35   thereby identify the compound which activates the GALR3
receptor.

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This invention provides a method of screening a plurality
of chemical compounds not known to inhibit the activation
of a GALR3 receptor to identify a compound which inhibits
the activation of the GALR3 receptor, which comprises (a)
5   preparing a cell extract from cells transfected with and
expressing DNA encoding the GALR3 receptor, isolating a
membrane fraction from the cell extract, contacting the
membrane fraction with the plurality of compounds in the
presence of a known GALR3 receptor agonist, under
10   conditions permitting activation of the GALR3 receptor;
(b) determining whether the activation of the GALR3
receptor is reduced in the presence of the plurality of
compounds, relative to the activation of the GALR3
receptor in the absence of the plurality of compounds;
15   and if so (c) separately determining the inhibition of
activation of the GALR3 receptor for each compound
included in the plurality of compounds, so as to thereby
identify the compound which inhibits the activation of
the GALR3 receptor.
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This invention provides a method of detecting expression
of a GALR3 receptor by detecting the presence of mRNA
coding for the GALR3 receptor which comprises obtaining
total mRNA from the cell and contacting the mRNA so
25   obtained with the above-described nucleic acid probe
under hybridizing conditions, detecting the presence of
mRNA hybridized to the probe, and thereby detecting the
expression of the GALR3 receptor by the cell.

30   This invention provides a method of treating an
abnormality in a subject, wherein the abnormality is
alleviated by the inhibition of a GALR3 receptor which
comprises administering to a subject an effective amount
of the above-described pharmaceutical composition
35   effective to decrease the activity of the GALR3 receptor
in the subject, thereby treating the abnormality in the
subject. In an embodiment, the abnormality is obesity.

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In another embodiment, the abnormality is bulimia.

This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by the activation of a GALR3 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition effective to activate the GALR3 receptor in the subject. In an embodiment, the abnormal condition is anorexia.

This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific human GALR3 receptor allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human GALR3 receptor and labeled with a detectable marker; (e) detecting labeled bands which have hybridized to DNA encoding a human GALR3 receptor labeled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) preparing DNA obtained for diagnosis by steps a-e; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

This invention provides a method of modifying feeding behavior of a subject which comprises administering to the subject an amount of a compound which is a galanin

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receptor agonist or antagonist effective to increase or decrease the consumption of food by the subject so as to thereby modify feeding behavior of the subject. In an embodiment, the compound is a GALR3 receptor antagonist

5   and the amount is effective to decrease the consumption of food by the subject. In another embodiment the compound is administered in combination with food.

In yet another embodiment the compound is a GALR3

10   receptor agonist and the amount is effective to increase the consumption of food by the subject. In a still further embodiment, the compound is administered in combination with food. In other embodiments the subject is a vertebrate, a mammal, a human or a canine.

15

This invention provides a process for determining whether a chemical compound is a GALR3 receptor agonist, which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR3 receptor,

20   isolating a membrane fraction from the cell extract, separately contacting the membrane fraction with both the chemical compound and GTPγS, and with only GTPγS, under conditions permitting the activation of the GALR3 receptor, and detecting GTPγS binding to the membrane

25   fraction, an increase in GTPγS binding in the presence of the compound indicating that the chemical compound activates the GALR3 receptor.

This invention provides a process for determining whether

30   a chemical compound is a GALR3 receptor antagonist, which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR3 receptor, isolating a membrane fraction from the cell extract, separately contacting the membrane fraction with the

35   chemical compound, GTPγS and a second chemical compound known to activate the GALR3 receptor, with GTPγS and only the second compound, and with GTPγS alone, under

conditions permitting the activation of the GALR3 receptor, detecting GTPγS binding to each membrane fraction, and comparing the increase in GTPγS binding in the presence of the compound and the second compound

5   relative to the binding of GTPγS alone, to the increase in GTPγS binding in the presence of the second chemical compound relative to the binding of GTPγS alone, a smaller increase in GTPγS binding in the presence of the compound and the second compound indicating that the

10   compound is a GALR3 receptor antagonist.

This invention further provides a process for identifying a chemical compound which specifically binds to a GALR3 receptor which comprises contacting cells containing DNA

15   encoding and expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the GALR3 receptor.

20

This invention also provides a process for identifying a chemical compound which specifically binds to a GALR3 receptor which comprises contacting a membrane fraction from a cell extract of cells containing DNA encoding and

25   expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the GALR3 receptor.

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This invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a GALR3 receptor which comprises

35   separately contacting cells expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with both the

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chemical compound and a second chemical compound known to
bind to the receptor, and with only the second chemical
compound, under conditions suitable for binding of both
compounds, and detecting specific binding of the chemical
5   compound to the GALR3 receptor, a decrease in the binding
of the second chemical compound to the GALR3 receptor in
the presence of the chemical compound indicating that the
chemical compound binds to the GALR3 receptor.

10   This invention further provides a process involving
competitive binding for identifying a chemical compound
which specifically binds to a human GALR3 receptor which
comprises separately contacting a membrane fraction from
a cell extract of cells expressing on their cell surface
15 the GALR3 receptor, wherein such cells do not normally
express the GALR3 receptor, with both the chemical
compound and a second chemical compound known to bind to
the receptor, and with only the second chemical compound,
under conditions suitable for binding of both compounds,
20 and detecting specific binding of the chemical compound
to the GALR3 receptor, a decrease in the binding of the
second chemical compound to the GALR3 receptor in the
presence of the chemical compound indicating that the
chemical compound binds to the GALR3 receptor.

25

This invention provides a method of screening a plurality
of chemical compounds not known to bind to a GALR3
receptor to identify a compound which specifically binds
to the GALR3 receptor, which comprises (a) preparing a
30 cell extract from cells transfected with and expressing
DNA encoding the GALR3 receptor, isolating a membrane
fraction from the cell extract, contacting the membrane
fraction with a compound known to bind specifically to
the GALR3 receptor; (b) contacting the preparation of step
35 (a) with the plurality of compounds not known to bind
specifically to the GALR3 receptor, under conditions
permitting binding of compounds known to bind the GALR3

receptor; (c) determining whether the binding of the
compound known to bind to the GALR3 receptor is reduced
in the presence of the compounds, relative to the binding
of the compound in the absence of the plurality of
5   compounds; and if so (d) separately determining the
binding to the GALR3 receptor of each compound included
in the plurality of compounds, so as to thereby identify
the compound which specifically binds to the GALR3
receptor.

10

This invention provides a method for determining whether
a compound is a GALR3 antagonist which comprises: (a)
administering to an animal a GALR3 agonist and measuring
the amount of food intake in the animal; (b)
15   administering to a second animal both the GALR3 agonist
and the compound, and measuring the amount of food intake
in the second animal; and (c) determining whether the
amount of food intake is reduced in the presence of the
compound relative to the amount of food intake in the
20   absence of the compound, so as to thereby determine
whether the compound is a GALR3 antagonist.

This invention provides a method of screening a plurality
of compounds to identify a compound which is a GALR3
25   antagonist which comprises: (a) administering to an
animal a GALR3 agonist and measuring the amount of food
intake in the animal; (b) administering to a second
animal the GALR3 agonist and at least one compound of the
plurality of compounds and measuring the amount of food
30   intake in the animal; (c) determining whether the amount
of food intake is reduced in the presence of at least one
compound of the plurality relative to the amount of food
intake in the absence of at least one compound of the
plurality, and if so; (d) separately determining whether
35   each compound is a GALR3 antagonist according to the
method of claim 118, so as to thereby identify a compound
which is a GALR3 antagonist.

This invention further provides a method of decreasing feeding behavior of a subject which comprises administering a compound which is a GALR3 receptor antagonist and a compound which is a Y5 receptor antagonist, the amount of such antagonists being effective to decrease the feeding behavior of the subject.

This invention provides a method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a GALR3 receptor agonist effective to decrease nociception in the subject.

This invention also provides a method of treating pain in a subject which comprises administering to the subject an amount of a compound which is a GALR3 receptor agonist effective to treat pain in the subject.

This invention further provides a method of treating diabetes in a subject which comprises administering to the subject an amount of a compound which is a GALR3 receptor antagonist effective to treat diabetes in the subject.

This invention also provides a process for determining whether a chemical compound specifically binds to and activates a GALR3 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with the chemical compound under conditions suitable for activation of the GALR3 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the

chemical compound indicating that the compound activates the GALR3 receptor.

This invention provides a process for determining whether
5   a chemical compound specifically binds to and inhibits activation of a GALR3 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the
10   GALR3 receptor, with both the chemical compound and a second chemical compound known to activate the GALR3 receptor, and with only the second chemical compound, under conditions suitable for activation of the GALR3 receptor, and measuring the second messenger response in
15   the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the
20   presence of only the second chemical compound indicating that the chemical compound inhibits activation of the. GALR3 receptor.

25   This invention provides a method of screening a plurality of chemical compounds not known to activate a GALR3 receptor to identify a compound which activates the GALR3 receptor which comprises: (a) contacting cells transfected with and expressing the GALR3 receptor with
30   the plurality of compounds not known to activate the GALR3 receptor, under conditions permitting activation of the GALR3 receptor; (b) determining whether the activity of the GALR3 receptor is increased in the presence of the compounds; and if so (c) separately determining
35   whether the activation of the GALR3 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates

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the GALR3 receptor.

This invention provides a method of screening a plurality
of chemical compounds not known to inhibit the activation
5   of a GALR3 receptor to identify a compound which inhibits
the activation of the GALR3 receptor, which comprises:
(a) contacting cells transfected with and expressing the
GALR3 receptor with the plurality of compounds in the
presence of a known GALR3 receptor agonist, under
10   conditions permitting activation of the GALR3 receptor;
(b) determining whether the activation of the GALR3
receptor is reduced in the presence of the plurality of
compounds, relative to the activation of the GALR3
receptor in the absence of the plurality of compounds;
15   and if so (c) separately determining the inhibition of
activation of the GALR3 receptor for each compound
included in the plurality of compounds, so as to thereby
identify the compound which inhibits the activation of
the GALR3 receptor.
20

This invention provides a process for determining whether
a chemical compound is a GALR3 receptor antagonist, which
comprises preparing a cell extract from cells transfected
with and expressing DNA encoding the GALR3 receptor,
25   isolating a membrane fraction from the cell extract,
separately contacting the membrane fraction with the
chemical compound, GTPγS and a second chemical compound
known to activate the GALR3 receptor, with GTPγS and
only the second compound, and with GTPγS alone, under
30   conditions permitting the activation of the GALR3
receptor, detecting GTPγS binding to each membrane
fraction, and comparing the increase in GTPγS binding in
the presence of the compound and the second compound
relative to the binding of GTPγS alone, to the increase
35   in GTPγS binding in the presence of the second chemical
compound relative to the binding of GTPγS alone, a
smaller increase in GTPγS binding in the presence of the

compound and the second compound indicating that the compound is a GALR3 receptor antagonist.

5

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Brief Description of the Figures

Figure 1 Nucleotide coding sequence of the rat hypothalamic galanin GALR3 receptor (Seq. I.D. No. 1), with partial 5' and 3' untranslated sequences. Start and stop codons are underlined.

Figure 2 Deduced amino acid sequence of the rat hypothalamic galanin GALR3 receptor (Seq. I.D. No. 2) encoded by the rat nucleotide sequence shown in Figure 1.

Figure 3 Nucleotide coding sequence of the human galanin GALR3 receptor (Seq. I.D. No. 3), with partial 5' and 3' untranslated sequences. Start and stop codons are underlined.

Figure 4 Deduced amino acid sequence of the human galanin GALR3 receptor (Seq. I.D. No. 4) encoded by the human nucleotide sequence shown in Figure 3. The nucleotide sequence shown in Figure 3 is translated from nucleotide 1 to the stop codon. Two possible starting methionines are underlined.

Figures 5A-5D Amino acid sequence alignment of the rat GALR3 receptor (top row) (Seq. ID No. 2), human GALR3 receptor (middle row) (Seq. ID No. 4) and rat GALR1 receptor (bottom row) (Seq. ID No. 5). Transmembrane domains (TM 1-7) are indicated by brackets above the sequence.

Figures 6A-6B Figure 6A: Long continuous trace (3 segments) demonstrates galanin responsivity and sensitivity to Ba^{++} block in an oocyte expressing hGalR3 and GIRK1 and GIRK4. Switching from ND96 to 1/2hK solution causes the appearance of a large resting (inward) K^+ current that increases further upon transient addition of 3 μM galanin. Subsequent addition of 300 μM

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Ba^{++} largely blocks both the resting and galanin-stimulated K$^+$ currents. After removal of Ba^{++} galanin responsivity is partially restored. **Figure 6B:** Concentration-response characteristic of a second oocyte expressing both hGalR3 and GIRKs. Stepwise increases in the concentration of porcine galanin from 10 to 10,000 nM result in a saturable increase in inward current.

Figure 7 Pertussis toxin sensitivity of GalR3 and GalR1 stimulation of GIRK currents. Normalized mean currents elicited by 0.1 μM (GalR1) and 1 μM (GalR3) galanin in oocytes injected 3 h prior with 2 ng of pertussis toxin compared to water-injected oocytes. For oocytes expressing GalR2 and α1a receptors, the response amplitude was measured as the peak of the Cl$^-$ current stimulated by 1 μM galanin or epinephrine, respectively. Number of observations appears in parenthesis below the x-axis. Apparent absence of a bar indicates an amplitude of 0 (no response above baseline).

Figures 8A-8G Concentration-response relations for 6 peptides at GalR3 receptors expressed in oocytes. **Figure 8A:** M32; **Figure 8B:** porcine galanin; **Figure 8C:** C7; **Figure 8D:** Gal -7-29; **Figure 8E:** Gal 1-16; **Figure 8F:** M40; **Figure 8G:** human galanin. Measurements of GIRK currents were made as shown for galanin in Fig. 6B. For all peptides, responses from 3-6 oocytes were averaged for each data point. Curves were fitted with the logistic equation $I = Imax/ (1 + (EC_{50} / [Agonist])^n)$, where EC_{50} is the concentration of agonist that produced half-maximal activation, and n the Hill coefficient. Fits were made with a Marquardt-Levenberg non-linear least-squares curve fitting algorithm.

Figures 9A-9B **Figure 9A:** Current-voltage relation for responses generated by galanin in oocytes expressing hGalR3, GIRK1 and GIRK4. Voltage ramps from -100 to +20

mV were applied at a rate of 50 mV/s. Ramps were generated in 1/2hK, 1/2hK + 1 μM galanin, and 1/2hK + galanin + 300 μM Ba^{++}. **Figure 9B**: the galanin-sensitive current (I_{gal}) was derived by subtracting the background current (1/2hK) from the galanin current (+gal); the total inward rectifier current (I_{tot}) was similarly obtained by subtracting the current in the presence of Ba^{++} from the galanin current. Both I_{gal} and I_{tot} display steep inward rectification and reverse at approximately -24 mV.

Figure 10

Autoradiograph demonstrating hybridization of radiolabeled rat GALR3 probe to RNA extracted from rat tissue in a solution hybridization/nuclease protection assay using ^{32}P labeled riboprobe. 2 μg of mRNA was used in each assay. The single band represents mRNA coding for the rat GALR3 receptor extracted from tissue indicated at the bottom of the gel. mRNA coding for the rGalR3 is present in: kidney, stomach, pancreas, pituitary, adrenal medulla, whole brain, hypothalamus, spinal cord, and medulla. Integrity of RNA was assessed using hybridization to mRNA coding to GAPDH. Biomax Film; 18 hr exposure, -70°C.

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Detailed Description of the Invention

Throughout this application, the following standard abbreviations are used to indicate specific nucleotide bases:

5   C=cytosine   A=adenine
       T=thymine   G=guanine

Furthermore, the term "agonist" is used throughout this application to indicate any peptide or non-peptidyl

10   compound which increases the activity of any of the receptors of the subject invention. The term "antagonist" is used throughout this application to indicate any peptide or non-peptidyl compound which decreases the activity of any of the receptors of the

15   subject invention.

The activity of a G-protein coupled receptor such as a galanin receptor may be measured using any of a variety of functional assays which are well-known in the art, in

20   which activation of the receptor in question results in an observable change in the level of some second messenger, including but not limited to adenylate cyclase, calcium mobilization, arachidonic acid release, ion channel activity, inositol phospholipid hydrolysis or

25   guanylyl cyclase. Heterologous expression systems utilizing appropriate host cells to express the nucleic acid of the subject invention are used to obtain the desired second messenger coupling. Receptor activity may also be assayed in an oocyte expression system, using

30   methods well known in the art.

This invention provides an isolated nucleic acid encoding a GALR3 galanin receptor. This invention further provides a recombinant nucleic acid encoding a GALR3

35   galanin receptor. In an embodiment, the galanin receptor is a vertebrate or a mammalian GALR3 receptor. In another embodiment, the galanin receptor is a rat GALR3

receptor. In another embodiment, the galanin receptor is a human GALR3 receptor. In an embodiment, the isolated nucleic acid encodes a receptor characterized by an amino acid sequence in the transmembrane region, which

5   has a homology of 70% or higher to the amino acid sequence in the transmembrane region of the rat galanin GALR3 receptor and a homology of less than 70% to the amino acid sequence in the transmembrane region of any GALR1 receptor. In an embodiment, the GALR3 receptor is

10   a rat GALR3 receptor. In another embodiment, the GALR3 receptor is a human GALR3 receptor.

This invention provides an isolated nucleic acid encoding a GALR3 receptor having the same or substantially the

15   same amino acid sequence as the amino acid sequence encoded by the plasmid K1086 (ATCC Accession No. 97747). In an embodiment, the nucleic acid is DNA. This invention further provides an isolated nucleic acid encoding a rat GALR3 receptor having the amino acid

20   sequence encoded by the plasmid K1086. This invention provides an isolated nucleic acid encoding a GALR3 receptor having substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No. 2). In another embodiment, the GALR3

25   receptor is the rat GALR3 receptor having the amino acid sequence shown in Figure 2 (Seq. ID NO. 2). In another embodiment, the nucleic acid comprises at least an intron. In still another embodiment, the nucleic acid comprises alternately spliced nucleic acid transcribed

30   from the nucleic acid contained in plasmid K1086. In an embodiment, the alternately spliced nucleic acid is mRNA transcribed from DNA encoding a galanin receptor.

In an embodiment, the GALR3 receptor is a human GALR3

35   receptor. This invention provides an isolated nucleic acid encoding a human GALR3 receptor having the same or substantially the same amino acid sequence as the amino

acid sequence encoded by plasmid pEXJ-hGalR3 (ATCC
Accession No. 97827). This invention provides an
isolated nucleic acid encoding a human GALR3 receptor,
wherein the human GALR3 receptor has a sequence, which
5   sequence comprises substantially the same amino acid
sequence as the sequence shown in Figure 4 (Seq. I.D. No.
4) from amino acid 60 through amino acid 427. In another
embodiment, the GALR3 receptor has a sequence, which
sequence comprises the sequence shown in Figure 4 (Seq.
10   ID NO. 4) from amino acid 60 through amino acid 427.

In another embodiment, the nucleic acid encoding the
human GALR3 receptor comprises an intron. In still
another embodiment, the nucleic acid encoding the human
15   GALR3 receptor comprises alternately spliced nucleic
acid.

The fact that introns are found in many G protein coupled
receptors raises the possibility that introns could exist
20   in coding or non-coding regions of GALR3; if so, a
spliced form of mRNA may encode additional amino acids
either upstream of the currently defined starting
methionine or within the coding region. Further, the
existence and use of alternative exons is possible,
25   whereby the mRNA may encode different amino acids within
the region comprising the exon. In addition, single
amino acid substitutions may arise via the mechanism of
RNA editing such that the amino acid sequence of the
expressed protein is different than that encoded by the
30   original gene (Burns et al., 1996; Chu et al., 1996).
Such variants may exhibit pharmacologic properties
differing from the receptor encoded by the original gene.

This invention provides a splice variant of the GALR3
35   receptors disclosed herein. This invention further
provides for alternate translation initiation sites and
alternately spliced or edited variants of nucleic acids

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encoding rat and human GALR3 receptors.

This invention provides the above-described isolated nucleic acid, wherein the nucleic acid is DNA. In one
5   embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In still another embodiment, the nucleic acid molecule is RNA. Methods for production and manipulation of nucleic acid molecules are well known in the art.
10

This invention provides a vector encoding the nucleic acid of human GALR3 receptor.

In another embodiment, the nucleic acid encodes a
15   vertebrate GALR3 receptor. In a separate embodiment, the nucleic acid encodes a mammalian GALR3 receptor. In another embodiment, the nucleic acid encodes a rat GALR3 receptor. In still another embodiment, the nucleic acid encodes a human GALR3 receptor.
20

This invention further provides nucleic acid which is degenerate with respect to the DNA comprising the coding sequence of the plasmid K1086 (ATCC Accession No. 97747). This invention further provides nucleic acid which is
25   degenerate with respect to any DNA encoding a GALR3 receptor. In an embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence of plasmid K1086, that is, a nucleotide sequence which is translated into the same
30   amino acid sequence. In an embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence of plasmid pEXJ-rGalR3T (ATCC Accession No. 97826). In another embodiment, the nucleic acid comprises a nucleotide
35   sequence which is degenerate with respect to the nucleotide sequence of plasmid pEXJ-hGalR3 (ATCC Accession No. 97827).

This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of the GALR3 galanin receptor, but which should not produce phenotypic changes. Alternatively, this invention also encompasses DNAs, cDNAs, and RNAs which hybridize to the DNA, cDNA, and RNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The nucleic acids of the subject invention also include nucleic acid molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

G-protein coupled receptors such as the GALR3 receptors of the present invention are characterized by the ability of an agonist to promote the formation of a high-affinity ternary complex between the agonist, the receptor, and an intracellular G-protein. This complex is formed in the presence of physiological concentrations of GTP, and results in the dissociation of the alpha subunit of the G protein from the beta and gamma subunits of the G protein, which further results in a functional response,

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i.e., activation of downstream effectors such as adenylyl cyclase or phospholipase C. This high-affinity complex is transient even in the presence of GTP, so that if the complex is destablized, the affinity of the receptor for
5   agonists is reduced.   Thus,   if a receptor is not optimally coupled to G protein under the conditions of an assay, an agonist will bind to the receptor with low affinity. In contrast, the affinity of the receptor for an antagonist is normally not significantly affected by
10   the presence or absence of G protein. Functional assays may be used to determine whether a compound binds to the receptor, but may be more time-consuming or difficult to perform than a binding assay. Therefore, it may desirable to produce a receptor which will bind to
15   agonists with high affinity in a binding assay. Examples of modified receptors which bind agonists with high affinity are disclosed in WO 96/14331, which describes neuropeptide Y receptors modified in the third intracellular domain. The modifications may include
20   deletions of 6-13 amino acids in the third intracellular loop. Such deletions preferably end immediately before the polar or charged residue at the beginning of helix six. In an embodiment, the deleted amino acids are at the carboxy terminus of the third intracellular domain.
25   Such modified receptors may be produced using methods well-known in the art such as site-directed mutagenesis or recombinant techniques using restriction enzymes.

This invention provides an isolated nucleic acid encoding
30   a modified GALR3 receptor, which differs from a GALR3 receptor by having an amino acid(s) deletion, replacement or addition in the third intracellular domain. In one embodiment, the modified GALR3 receptor differs by having a deletion in the third intracellular domain. In another
35   embodiment, the modified GALR3 receptor differs by having an amino acid replacement or addition to the third intracellular domain.

The modified receptors of this invention may be transfected into cells either transiently or stably using methods well-known in the art, examples of which are disclosed herein. This invention also provides for
5   binding assays using the modified receptors, in which the receptor is expressed either transiently or in stable cell lines. This invention further provides for a compound identified using a modified receptor in a binding assay such as the binding assays described
10   herein.

The nucleic acids described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products
15   for the large scale synthesis of the polypeptide by a variety of recombinant techniques. The nucleic acid molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful
20   methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

This invention also provides an isolated galanin GALR3 receptor protein. In one embodiment, the GALR3 receptor
25   protein has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid K1086. In another embodiment, the GALR3 receptor protein has the amino acid sequence encoded by plasmid K1086. In another embodiment, the protein has the amino acid
30   sequence encoded by the plasmid pEXJ-hGalR3. In an embodiment, the GALR3 receptor protein has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No. 2). In an embodiment, the GALR3 receptor comprises the same or
35   substantially the same amino acid sequence as the amino acid sequence shown in Figure 4 (Seq. I.D. No. 4) from amino acid 60 through amino acid 427.

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This invention provides a vector comprising the above-described nucleic acid molecule.

Vectors which comprise the isolated nucleic acid molecule
described hereinabove also are provided. Suitable
vectors comprise, but are not limited to, a plasmid or a
virus. These vectors may be transformed into a suitable
host cell to form a host cell expression system for the
production of a polypeptide having the biological
activity of a galanin GALR3 receptor. Suitable host
cells include, for example, neuronal cells such as the
glial cell line C6, a Xenopus cell such as an oocyte or
melanophore cell, as well as numerous mammalian cells and
non-neuronal cells.

This invention provides the above-described vector
adapted for expression in a bacterial cell which further
comprises the regulatory elements necessary for
expression of the nucleic acid in the bacterial cell
operatively linked to the nucleic acid encoding the GALR3
receptor as to permit expression thereof.

This invention provides the above-described vector
adapted for expression in a yeast cell which comprises
the regulatory elements necessary for expression of the
nucleic acid in the yeast cell operatively linked to the
nucleic acid encoding the GALR3 receptor as to permit
expression thereof.

This invention provides the above-described vector
adapted for expression in an insect cell which comprises
the regulatory elements necessary for expression of the
nucleic acid in the insect cell operatively linked to the
nucleic acid encoding the GALR3 receptor as to permit
expression thereof. In a still further embodiment, the
vector is a baculovirus.

This invention provides the above-described vector adapted for expression in a amphibian cell which further comprises the regulatory elements necessary for expression of the nucleic acid in the amphibian cell

5   operatively linked to the nucleic acid encoding the GALR3 receptor as to permit expression thereof.

In an embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements

10   necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the mammalian GALR3 receptor as to permit expression thereof.

15   In a further embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the rat GALR3 receptor as to

20   permit expression thereof.

In a still further embodiment, the vector is a plasmid.

In another embodiment, the plasmid is adapted for

25   expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the human GALR3 receptor as to permit expression thereof.

30
This invention provides the above-described plasmid adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of nucleic acid in a mammalian cell

35   operatively linked to the nucleic acid encoding the mammalian GALR3 receptor as to permit expression thereof.

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This invention provides a plasmid designated K1086 (ATCC Accession No. 97747) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the GALR3 galanin

5   receptor so as to permit expression thereof.

This plasmid (K1086) was deposited on October 8, 1996, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under

10   the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 97747.

15   This invention provides a plasmid designated pEXJ-hGalR3 (ATCC Accession No. 97827) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the human GALR3 galanin receptor so as to permit expression thereof.

20

This plasmid was deposited December 17, 1996, with the ATCC, 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A. under the provisions of the Budapest Treaty forth International Recognition of the Deposit of

25   Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 97827.

This invention provides a plasmid designated pEXJ-rGalR3T (ATCC Accession No. 97826) which comprises the regulatory

30   elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the rat GALR3 galanin receptor so as to permit expression thereof.

This plasmid was deposited December 17, 1996, with the

35   ATCC, 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of

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Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 97826.

5   This invention further provides for any vector or plasmid which comprises modified untranslated sequences, which are beneficial for expression in desired host cells or for use in binding or functional assays. For example, a vector or plasmid with untranslated sequences of varying lengths may express differing amounts of the receptor
10   depending upon the host cell used. In one embodiment, the vector or plasmid comprises the coding sequence of the GALR3 receptor and the regulatory elements necessary for expression in the host cell.

15   This invention provides a eukaryotic cell comprising the above-described plasmid or vector. This invention provides a mammalian cell comprising the above-described plasmid or vector. In an embodiment the cell is a Xenopus oocyte or melanophore cell. In an embodiment,
20   the cell is a neuronal cell such as the glial cell line C6. In an embodiment, the mammalian cell is non-neuronal in origin. In an embodiment, the mammalian cell is a COS-7 cell. In another embodiment the mammalian cell is a Chinese hamster ovary (CHO) cell. In another
25   embodiment, the cell is a mouse Y1 cell.

In still another embodiment, the mammalian cell is a 293 human embryonic kidney cell. In still another embodiment, the mammalian cell is a NIH-3T3 cell. In
30   another embodiment, the mammalian cell is an LM(tk-) cell.

In an embodiment, the mammalian cell is the 293 cell designated 293-rGALR3-105, which comprises the "trimmed"
35   plasmid pEXJ-rGalR3T. This cell line was deposited with the ATCC on February 19, 1997, under the provisions of the Budapest Treaty for the International Recognition of

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the Deposit of Microorganisms for the Purposes of Patent
Procedure, and was accorded ATCC Accession No. CRL-12287.

5   In an embodiment, the mammalian cell is the LM(tk-) cell
       designated L-hGALR3-228, which comprises the plasmid
       pEXJ-hGalR3. This cell line was deposited with the ATCC
       on June 25, 1997, under the provisions of the Budapest
       Treaty for the International Recognition of the Deposit
       of Microorganisms for the Purposes of Patent Procedure,
10   and was accorded ATCC Accession No. CRL-12373.

       This invention also provides an insect cell comprising
       the above-described vector. In an embodiment, the insect
       cell is an Sf9 cell. In another embodiment, the insect
15   cell is an Sf21 cell.

       This invention provides a membrane preparation isolated
       from any of the above-described cells.

20   This invention provides a nucleic acid probe comprising
       at   least   15   nucleotides,   which   probe   specifically
       hybridizes with a nucleic acid encoding a GALR3 receptor,
       wherein the probe has a unique sequence corresponding to
       a sequence present within one of the two strands of the
25   nucleic acid encoding the GALR3 receptor contained in
       plasmid K1086.

       This invention further provides a nucleic acid probe
       comprising   at   least   15   nucleotides,   which   probe
30   specifically hybridizes with a nucleic acid encoding a
       GALR3 receptor, wherein the probe has a unique sequence
       corresponding to a sequence present within one of the two
       strands of the nucleic acid encoding the GALR3 receptor
       contained in plasmid pEXJ-rGalR3T.

35
       This invention still further provides a nucleic acid
       probe comprising at least 15 nucleotides, which probe

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specifically hybridizes with a nucleic acid encoding a
GALR3 receptor, wherein the probe has a unique sequence
corresponding to a sequence present within (a) the
nucleic acid sequence shown in Figure 1 (Seq. ID NO. 1)
5   or (b) the reverse complement to the nucleic acid
sequence shown in Figure 1 (Seq. ID No. 1).

This invention also provides a nucleic acid probe
comprising at least 15 nucleotides, which probe
10   specifically hybridizes with a nucleic acid encoding a
GALR3 receptor, wherein the probe has a unique sequence
corresponding to a sequence present within one of the two
strands of the nucleic acid encoding the GALR3 receptor
contained in plasmid pEXJ-hGalR3.   This invention
15   provides a nucleic acid probe comprising at least 15
nucleotides, which probe specifically hybridizes with a
nucleic acid encoding a GALR3 receptor, wherein the probe
has a unique sequence corresponding to a sequence present
within (a) the nucleic acid sequence shown in Figure 3
20   (Seq. ID No. 3) or (b) the reverse complement to the
nucleic acid sequence shown in Figure 3 (Seq. ID NO. 3).

This invention provides a nucleic acid probe comprising
a nucleic acid which specifically hybridizes with a
25   nucleic acid encoding a GALR3 receptor, wherein the probe
comprises a unique sequence of at least 15 nucleotides
within a fragment of (a) the nucleic acid sequence
contained in plasmid K1086 or (b) the antisense nucleic
acid sequence capable of specifically hybridizing to the
30   nucleic acid sequence contained in plasmid K1086. In one
embodiment the GALR3 receptor is encoded by the coding
sequence of the plasmid K1086, or the reverse complement
(antisense sequence) of the coding sequence of plasmid
K1086.   In an embodiment, the nucleic acid encoding a
35   GALR3 receptor comprises an intron.

This invention further provides a nucleic acid probe

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comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a unique fragment of the sequence of a nucleic acid molecule encoding a GALR3 receptor. This invention also provides a nucleic

5   acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to the antisense sequence of a unique fragment of the sequence of a nucleic acid molecule encoding a GALR3 receptor.

10   In an embodiment, the nucleic acid probe is DNA. In another embodiment the nucleic acid probe is RNA. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to

15   form double-helical segments through hydrogen bonding between complementary base pairs.

This nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic

20   acid encoding the GALR3 galanin receptors can be used as a probe. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or

25   fluorescent dye, to facilitate detection of the probe. DNA probe molecules may be produced by insertion of a DNA molecule which encodes the GALR3 receptor into suitable vectors, such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells,

30   replication in the transformed bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA synthesizers.

35   RNA probes may be generated by inserting the DNA molecule which encodes the GALR3 galanin receptor downstream of a bacteriophage promoter such as T3, T7 or SP6. Large

amounts of RNA probe may be produced by incubating the labeled nucleotides with the linearized fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

5

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a GALR3 galanin receptor, so as to prevent translation of the mRNA.

10

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA molecule encoding a GALR3 receptor.

15   This invention provides an antisense oligonucleotide comprising chemical analogues of nucleotides.

This invention provides an antibody directed to a GALR3 receptor.   This invention also provides an antibody

20   directed to a rat GALR3 receptor.   This invention also provides an antibody directed to a human GALR3 receptor. In an embodiment, the rat GALR3 has an amino acid sequence substantially the same as an amino acid sequence encoded by plasmid K1086.   In an embodiment, the human

25   GALR3 receptor has a sequence, which sequence comprises substantially the same sequence as the sequence shown in Figure 4 (Seq. I.D. No. 4) from amino acid 60 through amino acid 427.   This invention further provides an antibody capable of competitively inhibiting the binding

30   of a second antibody to a GALR3 receptor.

This invention provides a monoclonal antibody directed to an epitope of a GALR3 receptor, which epitope is present

35   on the surface of a cell expressing a GALR3 receptor.

This invention provides a pharmaceutical composition

comprising an amount of the oligonucleotide effective to reduce activity of a GALR3 receptor by passing through a cell membrane and binding specifically with mRNA encoding a GALR3 receptor in the cell so as to prevent its

5   translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane. In an embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme.

10

This invention provides the above-described pharmaceutical composition, wherein the pharmaceutically acceptable carrier capable of passing through a cell membrane comprises a structure which binds to a receptor

15   specific for a selected cell type and is thereby taken up by cells of the selected cell type. In an emdodiment, the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

20

This invention provides a pharmaceutical composition comprising an amount of an antagonist effective to reduce the activity of a GALR3 receptor and a pharmaceutically acceptable carrier.

25

This invention provides a pharmaceutical composition comprising an amount of an agonist effective to increase activity of a GALR3 receptor and a pharmaceutically acceptable carrier.

30

This invention provides the above-described pharmaceutical composition which comprises an amount of the antibody effective to block binding of a ligand to the GALR3 receptor and a pharmaceutically acceptable

35   carrier.

As used herein, "pharmaceutically acceptable carriers"

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means any of the standard pharmaceutically acceptable
carriers. Examples include, but are not limited to,
phosphate buffered saline, physiological saline, water
and emulsions, such as oil/water emulsions.

5

This invention provides a transgenic nonhuman mammal
expressing DNA encoding a GALR3 receptor.

This invention provides a transgenic nonhuman mammal
10   comprising a homologous recombination knockout of the
native GALR3 receptor.

This invention provides a transgenic nonhuman mammal
whose genome comprises antisense DNA complementary to DNA
15   encoding a GALR3 receptor so placed as to be transcribed
into antisense mRNA which is complementary to mRNA
encoding a GALR3 receptor and which hybridizes to mRNA
encoding a GALR3 receptor thereby reducing its
translation.

20

This invention provides the above-described transgenic
nonhuman mammal, wherein the DNA encoding a GALR3
receptor additionally comprises an inducible promoter.

25   This invention provides the transgenic nonhuman mammal,
wherein the DNA encoding a GALR3 receptor additionally
comprises tissue specific regulatory elements.

In an embodiment, the transgenic nonhuman mammal is a
30   mouse.

Animal model systems which elucidate the physiological
and behavioral roles of GALR3 receptor are produced by
creating transgenic animals in which the activity of the
35   GALR3 receptor is either increased or decreased, or the
amino acid sequence of the expressed GALR3 receptor is
altered, by a variety of techniques. Examples of these

techniques include, but are not limited to: 1) Insertion
of normal or mutant versions of DNA encoding a GALR3
receptor, by microinjection, electroporation, retroviral
transfection or other means well known to those skilled
5   in the art, into appropriate fertilized embryos in order
to produce a transgenic animal or 2) Homologous
recombination of mutant or normal, human or animal
versions of these genes with the native gene locus in
transgenic animals to alter the regulation of expression
10   or the structure of these GALR3 receptor sequences. The
technique of homologous recombination is well known in
the art. It replaces the native gene with the inserted
gene and so is useful for producing an animal that cannot
express native GALR3 receptors but does express, for
15   example, an inserted mutant GALR3 receptor, which has
replaced the native GALR3 receptor in the animal's genome
by recombination, resulting in underexpression of the
transporter. Microinjection adds genes to the genome,
but does not remove them, and so is useful for producing
20   an animal which expresses its own and added GALR3
receptors, resulting in overexpression of the GALR3
receptors.

One means available for producing a transgenic animal,
25   with a mouse as an example, is as follows: Female mice
are mated, and the resulting fertilized eggs are
dissected out of their oviducts. The eggs are stored in
an appropriate medium such as M2 medium. DNA or cDNA
encoding a GALR3 receptor is purified from a vector by
30   methods well known in the art. Inducible promoters may
be fused with the coding region of the DNA to provide an
experimental means to regulate expression of the trans-
gene. Alternatively or in addition, tissue specific
regulatory elements may be fused with the coding region
35   to permit tissue-specific expression of the trans-gene.
The DNA, in an appropriately buffered solution, is put
into a microinjection needle (which may be made from

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capillary tubing using a pipet puller) and the egg to be
injected is put in a depression slide. The needle is
inserted into the pronucleus of the egg, and the DNA
solution is injected. The injected egg is then
5   transferred into the oviduct of a pseudopregnant mouse (a
mouse stimulated by the appropriate hormones to maintain
pregnancy but which is not actually pregnant), where it
proceeds to the uterus, implants, and develops to term.
As noted above, microinjection is not the only method for
10   inserting DNA into the egg cell, and is used here only
for exemplary purposes.

This invention provides a process for identifying a
chemical compound which specifically binds to a GALR3
15   receptor which comprises contacting cells containing DNA
encoding and expressing on their cell surface the GALR3
receptor, wherein such cells do not normally express the
GALR3 receptor, with the compound under conditions
suitable for binding, and detecting specific binding of
20   the chemical compound to the GALR3 receptor.

This invention further provides a process for identifying
a chemical compound which specifically binds to a GALR3
receptor which comprises contacting a membrane fraction
25   from a cell extract of cells containing DNA encoding and
expressing on their cell surface the GALR3 receptor,
wherein such cells do not normally express the GALR3
receptor, with the compound under conditions suitable for
binding, and detecting specific binding of the chemical
30   compound to the GALR3 receptor.

This invention also provides a process for determining
whether a chemical compound can specifically bind to a
GALR3 receptor which comprises contacting cells
35   transfected with and expressing DNA encoding the GALR3
receptor with the compound under conditions permitting
binding of compounds to such receptor, and detecting the

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presence of any such compound specifically bound to the
GALR3 receptor, so as to thereby determine whether the
ligand specifically binds to the GALR3 receptor.

5   This invention provides a process for determining whether
        a chemical compound can specifically bind to a GALR3
        receptor which comprises preparing a cell extract from
        cells transfected with and expressing DNA encoding the
        GALR3 receptor, isolating a membrane fraction from the
10   cell extract, contacting the membrane fraction with the
        compound under conditions permitting binding of compounds
        to such receptor, and detecting the presence of the
        compound specifically bound to the GALR3 receptor, so as
        to thereby determine whether the compound specifically
15   binds to the GALR3 receptor.

        In one embodiment, the GALR3 receptor is a mammalian
        GALR3 receptor.   In another embodiment, the GALR3
        receptor is a rat GALR3 receptor.   In still another
20   embodiment, the GALR3 receptor has the same or
        substantially the same amino acid sequence as that
        encoded by plasmid K1086.   In still another embodiment,
        the GALR3 receptor has the amino acid sequence encoded by
        plasmid K1086.   In another embodiment, the GALR3 receptor
25   has substantially the same amino acid sequence as the
        amino acid sequence shown in Figure 2 (Seq. ID NO. 2).
        In another embodiment, the GALR3 receptor has the amino
        acid sequence shown in Figure 2 (Seq. ID NO. 2).   In
        still another embodiment, the cells are transfected with
30   the plasmid pEXJ-RGALR3T (ATCC Accession No. 97826),
        encoding the rat GALR3 receptor.   Plasmid pEXJ-RGalR3T
        comprises the entire coding region of rat GALR3, but in
        which the 5' initiating ATG is joined directly to the
        vector, and which comprises only 100 nucleotides from the
35   3' untranslated region after the stop codon (i.e., up to
        and including nucleotide 1275 in Figure 1 (Seq. ID NO.
        1)).   Transfection of cells with the "trimmed" plasmid

results in a higher level of expression of the rat GALR3 receptor than the level of expression when plasmid K1086 is used. The use of the "trimmed" plasmid provides for greater convenience and accuracy in binding assays. In another embodiment the GALR3 receptor is a human GALR3 receptor. In still another embodiment, the GALR3 receptor has the same or substantially the same amino acid sequence as that encoded by plasmid pEXJ-hGalR3 (ATCC Accession No. 97827). In an embodiment, the human GALR3 receptor has a sequence, which sequence comprises substantially the same amino acid sequence as the sequence shown in Figure 4 (Seq. I.D. No. 4) from amino acid 60 through amino acid 427. In another embodiment, the GALR3 receptor has a sequence, which sequence comprises the sequence shown in Figure 4 (Seq. ID NO. 4) from amino acid 60 through amino acid 427.

In an embodiment, the above process further comprises determining whether the compound selectively binds to the GALR3 receptor relative to another galanin receptor. In another embodiment, the determination whether the compound selectively binds to the GALR3 receptor comprises: (a) determining the binding affinity of the compound for the GALR3 receptor and for such other galanin receptor; and (b) comparing the binding affinities so determined, the presence of a higher binding affinity for the GALR3 receptor than for such other galanin receptor indicating that the compound selectively binds to the GALR3 receptor. In one embodiment, the other galanin receptor is a GALR1 receptor. In another embodiment, the other galanin receptor is a GALR2 receptor.

This invention provides a process for determining whether a chemical compound is a GALR3 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the GALR3 receptor with the

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compound under conditions permitting the activation of
the GALR3 receptor, and detecting an increase in GALR3
receptor activity, so as to thereby determine whether the
compound is a GALR3 receptor agonist.

5

This invention provides a process for determining whether
a chemical compound is a GALR3 receptor agonist which
comprises preparing a cell extract from cells transfected
with and expressing DNA encoding the GALR3 receptor,

10   isolating a membrane fraction from the cell extract,
contacting the membrane fraction with the compound under
conditions permitting the activation of the GALR3
receptor, and detecting an increase in GALR3 receptor
activity, so as to thereby determine whether the compound

15   is a GALR3 receptor agonist.

In one embodiment, the GALR3 receptor is a rat GALR3
receptor. In another embodiment, the GALR3 receptor has
the same or substantially the same amino acid sequence as

20   that encoded by the plasmid K1086. In yet another
embodiment, the GALR3 receptor has the amino acid
sequence encoded by the plasmid K1086. In another
embodiment, the GALR3 receptor has substantially the same
amino acid sequence as the amino acid sequence shown in

25   Figure 2 (Seq. ID No. 2). In another embodiment, the
GALR3 receptor has the amino acid sequence shown in
Figure 2 (Seq. ID No. 2). In another embodiment, the
GALR3 receptor is a human GALR3 receptor. In still
another embodiment, the GALR3 receptor has the same or

30   substantially the same amino acid sequence as that
encoded by plasmid pEXJ-hGalR3 (ATCC Accession No.
97827). In another embodiment, the human GALR3 receptor
has a sequence, which sequence comprises substantially
the same amino acid sequence as the sequence shown in

35   Figure 4 (Seq. I.D. No. 4) from amino acid 60 through
amino acid 427. In another embodiment, the GALR3
receptor has a sequence, which sequence comprises the

sequence shown in Figure 4 (Seq. ID NO. 4) from amino acid 60 through amino acid 427. In another embodiment of this invention the cells are transfected with plasmid pEXJ-RGalR3T (ATCC Accession No. 97826).

This invention provides a process for determining whether a chemical compound is a GALR3 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the GALR3 receptor with the compound in the presence of a known GALR3 receptor agonist, such as galanin, under conditions permitting the activation of the GALR3 receptor, and detecting a decrease in GALR3 receptor activity, so as to thereby determine whether the compound is a GALR3 receptor antagonist.

This invention provides a process for determining whether a chemical compound is a GALR3 receptor antagonist which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR3 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known GALR3 receptor agonist, such as galanin, under conditions permitting the activation of the GALR3 receptor, and detecting a decrease in GALR3 receptor activity, so as to thereby determine whether the compound is a GALR3 receptor antagonist.

In an embodiment, the GALR3 receptor is a mammalian GALR3 receptor. In one embodiment of the invention, the GALR3 receptor is a rat GALR3 receptor. In another embodiment, the GALR3 receptor has the same or substantially the same amino acid sequence as that encoded by the plasmid K1086. In still another embodiment, the GALR3 receptor has the amino acid sequence encoded by the plasmid K1086. In another embodiment, the GALR3 receptor has substantially the same amino acid sequence as the amino acid sequence

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shown in Figure 2 (Seq. ID No. 2). In another
embodiment, the GALR3 receptor has the amino acid
sequence shown in Figure 2 (Seq. ID No. 2). In another
embodiment, the GALR3 receptor is a human GALR3 receptor.
5   In still another embodiment, the GALR3 receptor has the
same or substantially the same amino acid sequence as
that encoded by plasmid pEXJ-hGalR3 (ATCC Accession No.
97827). In another embodiment, the human GALR3 receptor
has a sequence, which sequence comprises substantially
10   the same amino acid sequence as the sequence shown in
Figure 4 (Seq. I.D. No. 4) from amino acid 60 through
amino acid 427. In another embodiment, the GALR3
receptor has a sequence, which sequence comprises the
sequence shown in Figure 4 (Seq. ID NO. 4) from amino
15   acid 60 through amino acid 427.

In an embodiment of the above-described methods, the cell
is a non-mammalian cell such as an insect cell or a
Xenopus cell. In another embodiment, the cell is a
20   mammalian cell. In a further embodiment, the cell is
non-neuronal in origin. In still further embodiments,
the non-neuronal cell is a COS-7 cell, 293 human
embryonic kidney cell, NIH-3T3 cell, a CHO cell, or
LM(tk-) cell. In another embodiment, the cell is a mouse
25   Y1 cell.

This invention provides a compound determined by the
above-described methods. In one embodiment of the above-
described methods, the compound is not previously known
30   to bind to a GALR3 receptor.

This invention provides a GALR3 agonist determined by the
above-described methods. This invention also provides a
GALR3 antagonist determined by the above-described
35   methods.

In an embodiment of any of the above processes, the cells

are transfected with and expressing GIRK1 and GIRK4.

In an embodiment of any of the above processes, the GALR3 receptor is a mammalian GALR3 receptor.

This invention provides a pharmaceutical composition which comprises an amount of a GALR3 receptor agonist determined by the above-described processes effective to increase activity of a GALR3 receptor and a pharmaceutically acceptable carrier. In an embodiment, the GALR3 receptor agonist is not previously known.

This invention provides a pharmaceutical composition which comprises an amount of a GALR3 receptor antagonist determined by the above-described processes effective to reduce activity of a GALR3 receptor and a pharmaceutically acceptable carrier. In an embodiment, the GALR3 receptor antagonist is not previously known.

This invention provides a pharmaceutical composition which comprises an amount of a GALR3 receptor agonist effective to increase activity of a GALR3 receptor and a pharmaceutically acceptable carrier.

This invention provides a pharmaceutical composition which comprises an amount of a GALR3 receptor antagonist effective to reduce activity of a GALR3 receptor and a pharmaceutically acceptable carrier.

In further embodiments of the above-described processes, the agonist or antagonist is not previously known to bind to a GALR3 receptor.

This invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a GALR3 receptor which comprises separately contacting cells expressing on their cell

surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the GALR3 receptor, a decrease in the binding of the second chemical compound to the GALR3 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GALR3 receptor.

This invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a human GALR3 receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the GALR3 receptor, a decrease in the binding of the second chemical compound to the GALR3 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GALR3 receptor.

This invention further provides a process for determining whether a chemical compound specifically binds to and activates a GALR3 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with the chemical compound under conditions suitable for activation of the GALR3 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in

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the second messenger response in the presence of the chemical compound indicating that the compound activates the GALR3 receptor.

5   This invention further provides a process for determining whether a chemical compound specifically binds to and activates a GALR3 receptor, which comprises contacting a membrane fraction from a cell extract of cells producing a second messenger response and expressing on

10   their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with the chemical compound under conditions suitable for activation of the GALR3 receptor, and measuring the second messenger response in the presence and in the

15   absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the GALR3 receptor.

20   In an embodiment of the above processes, the second messenger response comprises potassium channel activation and the change in second messenger is an increase in the level of inward potassium current.

25   In one embodiment of the above processes, the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a decrease in adenylate cyclase activity. In an embodiment, adenylate cyclase activity is determined by measurement

30   of cyclic AMP levels.

In another embodiment of the above processes, the second messenger response comprises arachidonic acid release and the change in second messenger response is an increase in

35   arachidonic acid levels.

In another embodiment of the above processes, the second

messenger response comprises intracellular calcium levels
and the change in second messenger response is an
increase in intracellular calcium levels.

5   In a still further embodiment of the above processes, the
second messenger response comprises inositol phospholipid
hydrolysis and the change in second messenger response is
an increase in inositol phospholipid hydrolysis.

10   This invention further provides a process for determining
whether a chemical compound specifically binds to and
inhibits activation of a GALR3 receptor, which comprises
separately contacting cells producing a second messenger
response and expressing on their cell surface the GALR3
15   receptor, wherein such cells do not normally express the
GALR3 receptor, with both the chemical compound and a
second chemical compound known to activate the GALR3
receptor, and with only the second compound, under
conditions suitable for activation of the GALR3 receptor,
20   and measuring the second messenger response in the
presence of only the second chemical compound and in the
presence of both the second chemical compound and the
chemical compound, a smaller change in the second
messenger response in the presence of both the chemical
25   compound and the second chemical compound than in the
presence of only the second chemical compound indicating
that the chemical compound inhibits activation of the
GALR3 receptor.

30   This invention further provides a process for determining
whether a chemical compound specifically binds to and
inhibits activation of a GALR3 receptor, which comprises
separately contacting a membrane fraction from a cell
extract of cells producing a second messenger response
35   and expressing on their cell surface the GALR3 receptor,
wherein such cells do not normally express the GALR3
receptor, with both the chemical compound and a second

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chemical compound known to activate the GALR3 receptor, and with only the second chemical compound, under conditions suitable for activation of the GALR3 receptor, and measuring the second messenger response in the

5   presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the

10 presence of only the second chemical compound indicating that the chemical compound inhibits activation of the GALR3 receptor.

In an embodiment of the above processes, the second

15 messenger response comprises potassium channel activation and the change in second messenger response is a smaller increase in the level of inward potassium current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second

20 chemical compound.

In one embodiment of the above processes, the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a smaller

25 decrease in the level of adenylate cyclase activity in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In an embodiment, adenylate cyclase activity is determined by measurement of cyclic AMP

30 levels.

In another embodiment of the above processes the second messenger response comprises arachidonic acid release, and the change in second messenger response is a smaller

35 increase in arachidonic acid levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical

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compound.

In another embodiment of the above processes the second
messenger response comprises intracellular calcium
5   levels, and the change in second messenger response is a
smaller increase in intracellular calcium levels in the
presence of both the chemical compound and the second
chemical compound than in the presence of only the second
chemical compound.
10

In yet another embodiment of the above processes, the
second messenger response comprises inositol phospholipid
hydrolysis, and the change in second messenger response
is a smaller increase in inositol phospholipid hydrolysis
15   in the presence of both the chemical compound and the
second chemical compound than in the presence of only the
second chemical compound.

In an embodiment of any of the above processes, the GALR3
20   receptor is a mammalian GALR3 receptor. In another
embodiment of the above processes, the GALR3 receptor is
a rat GALR3 receptor or a human GALR3 receptor. In still
another embodiment of the above processes, the GALR3
receptor has the same or substantially the same amino
25   acid sequence as encoded by the plasmid K1086 (ATCC
Accession No. 97747). In another embodiment, the GALR3
receptor has substantially the same amino acid sequence
as the amino acid sequence shown in Figure 2 (Seq. ID No.
2). In another embodiment, the GALR3 receptor has the
30   amino acid sequence shown in Figure 2 (Seq. ID No. 2).
In still another embodiment, the GALR3 receptor has the
same or substantially the same amino acid sequence as
that encoded by plasmid pEXJ-hGalR3 (ATCC Accession No.
97827). In another embodiment, the human GALR3 receptor
35   has a sequence, which sequence comprises substantially
the same amino acid sequence as the sequence shown in
Figure 4 (Seq. I.D. No. 4) from amino acid 60 through

amino acid 427. In another embodiment, the GALR3 receptor has a sequence, which sequence comprises the sequence shown in Figure 4 (Seq. ID NO. 4) from amino acid 60 through amino acid 427. In another embodiment of this invention the cells are transfected with plasmid pEXJ-RGalR3T (ATCC Accession No. 97826).

In one embodiment of the above-described processes, the cell is a non-mammalian cell such as an insect cell or a *Xenopus* cell. In another embodiment of any of the above processes, the cell is a mammalian cell. In still further embodiments, the cell is nonneuronal in origin. In another embodiment of the above processes, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, CHO cell, mouse Y1 cell, NIH-3T3 cell or LM(tk-) cell.

This invention further provides a compound determined by any of the above processes. In another embodiment, the compound is not previously known to bind to a GALR3 receptor.

This invention provides a pharmaceutical composition which comprises an amount of a GALR3 receptor agonist determined by any of the above processes effective to increase activity of a GALR3 receptor and a pharmaceutically acceptable carrier. In an embodiment, the GALR3 receptor agonist is not previously known.

This invention provides a pharmaceutical composition which comprises an amount of a GALR3 receptor antagonist determined by any of the above processes effective to reduce activity of a GALR3 receptor and a pharmaceutically acceptable carrier. In an embodiment, the GALR3 receptor antagonist is not previously known.

This invention provides a method of screening a plurality

of chemical compounds not known to bind to a GALR3
receptor to identify a compound which specifically binds
to the GALR3 receptor, which comprises (a) contacting
cells transfected with and expressing DNA encoding the
5   GALR3 receptor with a compound known to bind specifically
to the GALR3 receptor; (b) contacting the preparation of
step (a) with the plurality of compounds not known to
bind specifically to the GALR3 receptor, under conditions
permitting binding of compounds known to bind the GALR3
10   receptor; (c) determining whether the binding of the
compound known to bind to the GALR3 receptor is reduced
in the presence of the compounds, relative to the binding
of the compound in the absence of the plurality of
compounds; and if so (d) separately determining the
15   binding to the GALR3 receptor of each compound included
in the plurality of compounds, so as to thereby identify
the compound which specifically binds to the GALR3
receptor.

20   This invention provides a method of screening a plurality
of chemical compounds not known to bind to a GALR3
receptor to identify a compound which specifically binds
to the GALR3 receptor, which comprises (a) preparing a
cell extract from cells transfected with and expressing
25   DNA encoding the GALR3 receptor, isolating a membrane
fraction from the cell extract, contacting the membrane
fraction with a compound known to bind specifically to
the GALR3 receptor; (b) contacting the preparation of
step (a) with the plurality of compounds not known to
30   bind specifically to the GALR3 receptor, under conditions
permitting binding of compounds known to bind the GALR3
receptor; (c) determining whether the binding of the
compound known to bind to the GALR3 receptor is reduced
in the presence of the compounds, relative to the binding
35   of the compound in the absence of the plurality of
compounds; and if so (d) separately determining the
binding to the GALR3 receptor of each compound included

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in the plurality of compounds, so as to thereby identify the compound which specifically binds to the GALR3 receptor.

5   In an embodiment of any of the above processes, the GALR3 receptor is a mammalian GALR3 receptor. In an embodiment of the above-described methods, the GALR3 receptor is a rat GALR3 receptor. In another embodiment, the GALR3 receptor has the same or substantially the same amino
10   acid sequence as the amino acid sequence encoded by plasmid K1086. In another embodiment, the GALR3 receptor has substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. ID NO. 2). In another embodiment, the GALR3 receptor has the amino
15   acid sequence shown in Figure 2 (Seq. ID No. 2). In another embodiment, the GALR3 receptor is a human GALR3 receptor. In still another embodiment, the GALR3 receptor has the same or substantially the same amino acid sequence as that encoded by plasmid pEXJ-hGalR3
20   (ATCC Accession No. 97827). In another embodiment, the human GALR3 receptor has a sequence, which sequence comprises substantially the same amino acid sequence as the sequence shown in Figure 4 (Seq. I.D. No. 4) from amino acid 60 through amino acid 427. In another
25   embodiment, the GALR3 receptor has a sequence, which sequence comprises the sequence shown in Figure 4 (Seq. ID NO. 4) from amino acid 60 through amino acid 427.

       This invention provides a method of screening a plurality
30   of chemical compounds not known to activate a GALR3 receptor to identify a compound which activates the GALR3 receptor which comprises (a) contacting cells transfected with and expressing the GALR3 receptor with the plurality of compounds not known to activate the GALR3 receptor,
35   under conditions permitting activation of the GALR3 receptor; (b) determining whether the activity of the GALR3 receptor is increased in the presence of the

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compounds; and if so (c) separately determining whether the activation of the GALR3 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the GALR3

5   receptor.

This invention provides a method of screening a plurality of chemical compounds not known to activate a GALR3 receptor to identify a compound which activates the GALR3

10   receptor which comprises (a) preparing a cell extract from cells transfected with and expressing DNA encoding the GALR3 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the plurality of compounds not known to activate the

15   GALR3 receptor, under conditions permitting activation of the GALR3 receptor; (b) determining whether the activity of the GALR3 receptor is increased in the presence of the compounds; and if so (c) separately determining whether the activation of the GALR3 receptor is increased by each

20   compound included in the plurality of compounds, so as to thereby identify the compound which activates the GALR3 receptor.

In an embodiment of the above processes, the cells are

25   transfected with and expressing GIRK1 and GIRK4.   In another embodiment, the GALR3 receptor is a mammalian GALR3 receptor.

In an embodiment of any of the above-described methods,

30   the GALR3 receptor is a rat GALR3 receptor.   In still another embodiment, the GALR3 receptor has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid K1086.   In another embodiment, the GALR3 receptor has substantially the same

35   amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. ID No. 2).   In another embodiment, the GALR3 receptor has the amino acid sequence shown in

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Figure 2 (Seq. ID No. 2). In another embodiment, the GALR3 receptor is a human GALR3 receptor. In still another embodiment, the GALR3 receptor has the same or substantially the same amino acid sequence as that

5　encoded by plasmid pEXJ-hGalR3 (ATCC Accession No. 97827). In another embodiment, the human GALR3 receptor has a sequence, which sequence comprises substantially the same amino acid sequence as the sequence shown in Figure 4 (Seq. I.D. No. 4) from amino acid 60 through

10　amino acid 427. In another embodiment, the GALR3 receptor has a sequence, which sequence comprises the sequence shown in Figure 4 (Seq. ID NO. 4) from amino acid 60 through amino acid 427.

15　This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a GALR3 receptor to identify a compound which inhibits the activation of the GALR3 receptor, which comprises (a) contacting cells transfected with and expressing the

20　GALR3 receptor with the plurality of compounds in the presence of a known GALR3 receptor agonist, under conditions permitting activation of the GALR3 receptor; (b) determining whether the activation of the GALR3 receptor is reduced in the presence of the plurality of

25　compounds, relative to the activation of the GALR3 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the GALR3 receptor for each compound included in the plurality of compounds, so as to thereby

30　identify the compound which inhibits the activation of the GALR3 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation

35　of a GALR3 receptor to identify a compound which inhibits the activation of the GALR3 receptor, which comprises (a) preparing a cell extract from cells transfected with and

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expressing DNA encoding the GALR3 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the plurality of compounds in the presence of a known GALR3 receptor agonist, under

5   conditions permitting activation of the GALR3 receptor; (b) determining whether the activation of the GALR3 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GALR3 receptor in the absence of the plurality of compounds;

10   and if so (c) separately determining the inhibition of activation of the GALR3 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the GALR3 receptor.

15

In an embodiment of the above processes, the cells are transfected with and expressing GIRK1 and GIRK4. In another embodiment, the GALR3 receptor is a mammalian GALR3 receptor.

20

In an embodiment of any of the above-described methods, the GALR3 receptor is a rat GALR3 receptor. In another embodiment, the GALR3 receptor has the same or substantially the same amino acid sequence as the amino

25   acid sequence encoded by plasmid K1086. In another embodiment, the GALR3 receptor has substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. ID No. 2). In another embodiment, the GALR3 receptor has the amino acid sequence shown in

30   Figure 2 (Seq. ID No. 2). In another embodiment, the GALR3 receptor is a human GALR3 receptor. In still another embodiment, the GALR3 receptor has the same or substantially the same amino acid sequence as that encoded by plasmid pEXJ-hGalR3 (ATCC Accession No.

35   97827). In another embodiment, the human GALR3 receptor has a sequence, which sequence comprises substantially the same amino acid sequence as the sequence shown in

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Figure 4 (Seq. I.D. No. 4) from amino acid 60 through amino acid 427. In another embodiment, the GALR3 receptor has a sequence, which sequence comprises the sequence shown in Figure 4 (Seq. ID NO. 4) from amino

5   acid 60 through amino acid 427.

In an embodiment of the above processes, the cells are transfected with and expressing GIRK1 and GIRK4. In an embodiment of the above processes, receptor activation is

10   determined by measurement of potassium channel activation. In an embodiment, receptor activation is determined by measurement of an increase in inward potassium current. In another embodiment, inhibition of receptor activation is determined by a smaller increase

15   in inward potassium current in the presence of the compound and a galanin receptor agonist than in the presence of only the galanin receptor agonist. In an embodiment, the galanin receptor agonist is galanin.

20   This invention provides a pharmaceutical composition comprising a compound identified by any of the above-described methods effective to increase GALR3 receptor activity and a pharmaceutically acceptable carrier.

25   This invention provides a pharmaceutical composition comprising a compound identified by any of the above-described methods effective to decrease GALR3 receptor activity and a pharmaceutically acceptable carrier.

30   This invention provides any of the above processes, which further comprises a process for determining whether the compound selectively activates the GALR3 receptor relative to another galanin receptor.

35   This invention provides a process for determining whether a compound selectively activates the GALR3 receptor relative to another galanin receptor which comprises: (a)

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determining the potency of the compound for the GALR3 receptor and for such other galanin receptor; and (b) comparing the potencies so determined, the presence of a higher potency for the GALR3 receptor than for such other galanin receptor indicating that the compound selectively activates the GALR3 receptor. In an embodiment of the above process such other galanin receptor is a GALR1 receptor. In another embodiment, such other galanin receptor is a GALR2 receptor.

This invention further provides any of the above processes, which further comprises a process for determining whether the compound selectively inhibits the activation of the GALR3 receptor relative to another galanin receptor.

This invention provides a process for determining whether a compound selectively inhibits the activation of the GALR3 receptor relative to another galanin receptor, which comprises: (a) determining the decrease in the potency of a known galanin receptor agonist for the GALR3 receptor in the presence of the compound, relative to the potency of the agonist in the absence of the compound; (b) determining the decrease in the potency of the agonist for such other galanin receptor in the presence of the compound, relative to the potency of the agonist in the absence of the compound; and (c) comparing the decrease in potencies so determined, the presence of a greater decrease in potency for the GALR3 receptor than for such other galanin receptor indicating that the compound selectively inhibits the activation of the GALR3 receptor. In an embodiment of the above processes, such other galanin receptor is a GALR1 receptor. In another embodiment, such other galanin receptor is a GALR2 receptor.

In an embodiment of any of the above-described methods,

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the activation of the GALR3 receptor is determined by a
second messenger assay. In an embodiment, the second
messenger assay measures adenylate cyclase activity. In
other embodiments, the second messenger is cyclic AMP,
5   intracellular   calcium,   or   arachidonic   acid   or   a
phosphoinositol lipid metabolite. Receptor activation
may also be measured by assaying the binding of GTPγS
(gamma thiol GTP) to membranes, which precedes and is
therefore independent of second messenger coupling.
10

This invention provides a process for determining whether
a chemical compound is a GALR3 receptor agonist, which
comprises preparing a cell extract from cells transfected
with and expressing DNA encoding the GALR3 receptor,
15   isolating a membrane fraction from the cell extract,
separately contacting the membrane fraction with both the
chemical compound and GTPγS, and with only GTPγS, under
conditions permitting the activation of the GALR3
receptor, and detecting GTPγS binding to the membrane
20   fraction, an increase in GTPγS binding in the presence of
the compound indicating that the chemical compound
activates the GALR3 receptor.

This invention provides a process for determining whether
25   a chemical compound is a GALR3 receptor antagonist, which
comprises preparing a cell extract from cells transfected
with and expressing DNA encoding the GALR3 receptor,
isolating a membrane fraction from the cell extract,
separately contacting the membrane fraction with the
30   chemical compound, GTPγS and a second chemical compound
known to activate the GALR3 receptor, with GTPγS and
only the second compound, and with GTPγS alone, under
conditions permitting the activation of the GALR3
receptor, detecting GTPγS binding to each membrane
35   fraction, and comparing the increase in GTPγS binding in
the presence of the compound and the second compound
relative to the binding of GTPγS alone, to the increase

in GTPγS binding in the presence of the second chemical compound relative to the binding of GTPγS alone, a smaller increase in GTPγS binding in the presence of the compound and the second compound indicating that the compound is a GALR3 receptor antagonist.

In an embodiment of any of the above-described processes, the second chemical compound is a labeled compound. In another embodiment, the second chemical compound is a radiolabeled compound.

In an embodiment of any of the above-described processes, the GALR3 receptor is a mammalian GALR3 receptor. In another embodiment of any of the above-described processes, the GALR3 receptor has substantially the same amino acid sequence as encoded by the plasmid K1086 (ATCC Accession No. 97747). In another embodiment of any of the above-described processes, the GALR3 receptor has substantially the same amino acid sequence as that shown in Figure 2 (Seq. ID No. 2). In still another embodiment of any of the above-described processes, the GALR3 receptor has substantially the same amino acid sequence as encoded by the plasmid pEXJ-hGalR3 (ATCC Accession No. 97827). In an embodiment of any of the above-described processes, the GALR3 receptor has a sequence, which sequence comprises substantially the same amino acid sequence as that shown in Figure 4 (Seq. ID No. 4) from amino acid 60 through amino acid 427. In still another embodiment of any of the above-described processes, the GALR3 receptor has a sequence, which sequence comprises a sequence shown in Figure 4 (Seq. ID No. 4) from amino acid 60 through amino acid 427.

In an embodiment of any of the above-described processes, the cell is an insect cell.

In an embodiment of any of the above-described processes, the cell is a mammalian cell. In another embodiment of

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any of the above-described processes, the mammalian cell
is nonneuronal in origin.   In another embodiment of any
of the above-described processes, the nonneuronal cell is
a COS-7 cell, CHO cell, 293 human embryonic kidney cell,
5   NIH-3T3 cell or LM(tk-) cell.   In another embodiment, the
nonneuronal cell is the 293 human embryonic kidney cell
designated 293-rGALR3-105 (ATCC Accession No. CRL-12287).
In still another embodiment, the nonneuronal cell is the
LM(tk-) cell designated L-hGALR3-228 (ATCC Accession No.
10   CRL-12373).

GTPγS assays are well-known in the art, and it is
expected that variations on the method described above,
such as are described by e.g., Tian et al.   (1994)   or
15   Lazareno and Birdsall (1993), may be used by one of
ordinary skill in the art.   In an embodiment of any of
the above-described processes, the compound is not
previously known to bind to a GALR3 receptor.   This
invention also provides a compound determined by any of
20   the above-described processes.

This invention further provides a method of measuring
GALR3 receptor activation in an oocyte expression system
such as a Xenopus oocyte or melanophore.   In an
25   embodiment, receptor activation is determined by
measurement of ion channel activity, e.g., using the
voltage clamp technique (Stühmer, 1992).   In an
embodiment, receptor activation is determined by the
measurement of inward potassium current.   In the
30   experiments described hereinbelow, receptor activation
was determined by measurement of inward potassium current
in the presence of elevated external potassium levels.
However, this invention also provides a method of
determining GALR3 receptor activation by measurement of
35   outward potassium current in the presence of low (i.e.,
physiologic) external potassium levels, using similar
methods, which are well-known in the art.

Expression of genes in *Xenopus* oocytes is well known in the art (A. Coleman, <u>Transcription and Translation: A Practical Approach</u> (B.D. Hanes, S.J. Higgins, eds., pp 271-302, IRL Press, Oxford, 1984; Y. Masu et al., <u>Nature</u>
5   329:21583-21586, 1994) and is performed using microinjection of native mRNA or <u>in vitro</u> synthesized mRNA into frog oocytes. The preparation of <u>in vitro</u> synthesized mRNA can be performed by various standard techniques (J. Sambrook et al., <u>Molecular Cloning: A</u>
10   <u>Laboratory Manual</u>, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989) including using T7 polymerase with the mCAP RNA capping kit (Stratagene). The use of DNA vectors that include 5' and 3' untranslated (UT) regions of *Xenopus* β-globin gene
15   flanking the coding region of the gene of interest has been found to increase the level of expression in *Xenopus* oocytes (Linman, et al., 1992).

       In an embodiment of any of the above-described processes
20   or methods, the cell is a non-mammalian cell such as an insect cell or *Xenopus* cell. In a further embodiment of the invention, the cell is a mammalian cell. In another embodiment of the invention, the mammalian cell is non-neuronal in origin. In still further embodiments of the
25   invention, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a mouse Y1 cell, a CHO cell, or an NIH-3T3 cell.

       This invention provides a pharmaceutical composition
30   comprising a compound identified by the above-described methods and a pharmaceutically acceptable carrier.

       In an embodiment of the above-described methods, the cell is non-neuronal in origin. In a further embodiment, the
35   non-neuronal cell is a COS-7 cell, 293 human embryonic kidney cell, CHO cell, NIH-3T3 cell or LM(tk-) cell.

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In one embodiment of the above-described methods, the compound is not previously known to bind to a GALR3 receptor.

5   This invention provides a GALR3 receptor agonist detected by the above-described methods. This invention provides a GALR3 receptor antagonist detected by the above-described methods. In an embodiment the cell is a non-mammalian cell, for example, a Xenopus oocyte or
10   melanophore. In another embodiment the cell is a neuronal cell, for example, a glial cell line such as C6. In an embodiment, the cell is non-neuronal in origin. In a further embodiment, the cell is a Cos-7 or a CHO cell, a 293 human embryonic kidney cell, an LM(tk-) cell or an
15   NIH-3T3 cell.

This invention provides a pharmaceutical composition comprising a drug candidate identified by the above-described methods and a pharmaceutically acceptable
20   carrier.

This invention provides a method for determining whether a chemical compound is a GALR3 antagonist which comprises: (a) administering to an animal a GALR3 agonist
25   and measuring the amount of food intake in the animal; (b) administering to a second animal both the GALR3 agonist and the chemical compound, and measuring the amount of food intake in the second animal; and (c) determining whether the amount of food intake is reduced
30   in the presence of the chemical compound relative to the amount of food intake in the absence of the compound, so as to thereby determine whether the compound is a GALR3 antagonist.

35   This invention further provides a method of screening a plurality of chemical compounds to identify a chemical compound which is a GALR3 antagonist which comprises: (a)

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administering to an animal a GALR3 agonist and measuring
the amount of food intake in the animal; (b)
administering to a second animal the GALR3 agonist and at
least one chemical compound of the plurality of
compounds, and measuring the amount of food intake in the
animal; (c) determining whether the amount of food intake
is reduced in the presence of at least one chemical
compound of the plurality of chemical compounds relative
to the amount of food intake in the absence of at least
one of the compounds, and if so; (d) separately
determining whether each chemical compound is a GALR3
antagonist according to the method described above, so as
to thereby determine if the chemical compound is a GALR3
antagonist.   In another embodiment the animal is a non-
human mammal.   In a further embodiment, the animal is a
rodent.

This invention provides a method of detecting expression
of a GALR3 receptor by detecting the presence of mRNA
coding for the GALR3 receptor which comprises obtaining
total mRNA from a cell or tissue sample and contacting
the mRNA so obtained with the above-described nucleic
acid probe under hybridizing conditions, detecting the
presence of mRNA hybridized to the probe, and thereby
detecting the expression of the GALR3 receptor by the
cell or in the tissue.

This invention provides a method of treating an
abnormality in a subject, wherein the abnormality is
alleviated by administering to the subject an amount of
a GALR3 selective compound, effective to treat the
abnormality. Abnormalities which may be treated include
cognitive disorder, pain, sensory disorder (olfactory,
visual), motor coordination abnormality, motion sickness,
neuroendocrine disorders, sleep disorders, migraine,
Parkinson's disease, hypertension, heart failure,
convulsion/epilepsy, traumatic brain injury, diabetes,

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glaucoma, electrolyte imbalances, respiratory disorders
(asthma, emphysema), depression, reproductive disorders,
gastric and intestinal ulcers, gastroesophageal reflux
disorder, gastric hypersecretion, gastrointestinal
5   motility disorders (diarrhea), inflammation, immune
disorders, and anxiety. In one embodiment the compound
is an agonist. In another embodiment the compound is an
antagonist.

10   This invention provides a method of treating an
abnormality in a subject, wherein the abnormality is
alleviated by the inhibition of a GALR3 receptor which
comprises administering to a subject an effective amount
of the above-described pharmaceutical composition
15   effective to decrease the activity of the GALR3 receptor
in the subject, thereby treating the abnormality in the
subject. In an embodiment, the abnormality is obesity.
In another embodiment, the abnormality is bulimia.

20   This invention provides a method of treating an
abnormality in a subject wherein the abnormality is
alleviated by the activation of a GALR3 receptor which
comprises administering to a subject an effective amount
of the above-described pharmaceutical composition
25   effective to activate the GALR3 receptor in the subject.
In an embodiment, the abnormal condition is anorexia.

In another embodiment, the compound binds selectively to
a GALR3 receptor. In yet another embodiment, the
30   compound binds to the GALR3 receptor with an affinity
greater than ten-fold higher than the affinity with which
the compound binds to a GALR1 receptor. In a still
further embodiment, the compound binds to the GALR3
receptor with an affinity greater than ten-fold higher
35   than the affinity with which the compound binds to a
GALR2 receptor.

This invention provides a method of detecting the presence of a GALR3 receptor on the surface of a cell which comprises contacting the cell with the above-described antibody under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a GALR3 receptor on the surface of the cell.

This invention provides a method of determining the physiological effects of varying levels of activity of GALR3 receptors which comprises producing a transgenic nonhuman mammal whose levels of GALR3 receptor activity are varied by use of an inducible promoter which regulates GALR3 receptor expression.

This invention provides a method of determining the physiological effects of varying levels of activity of GALR3 receptors which comprises producing a panel of transgenic nonhuman mammals each expressing a different amount of GALR3 receptor.

This invention provides a method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a GALR3 receptor comprising administering a compound to the above-described transgenic nonhuman mammal and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overactivity of a GALR3 receptor, the alleviation of the abnormality identifying the compound as an antagonist.

This invention provides an antagonist identified by the above-described methods. This invention provides a pharmaceutical composition comprising an antagonist identified by the above-described methods and a pharmaceutically acceptable carrier.

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This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a GALR3 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition, thereby treating the abnormality.

This invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GALR3 receptor comprising administering a compound to a transgenic nonhuman mammal and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal, the alleviation of the abnormality identifying the compound as an agonist.

This invention provides an agonist identified by the above-described methods.

This invention provides a pharmaceutical composition comprising an agonist identified by the above-described methods and a pharmaceutically acceptable carrier.

This invention provides a method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GALR3 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition, thereby treating the abnormality.

This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific human GALR3 receptor allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c)

electrophoretically separating the resulting DNA
fragments on a sizing gel; (d) contacting the resulting
gel with a nucleic acid probe capable of specifically
hybridizing with a unique sequence included within the
sequence of a nucleic acid molecule encoding a human
GALR3 receptor and labelled with a detectable marker; (e)
detecting labelled bands which have hybridized to DNA
encoding a human GALR3 receptor labelled with a
detectable marker to create a unique band pattern
specific to the DNA of subjects suffering from the
disorder; (f) preparing DNA obtained for diagnosis by
steps a-e; and (g) comparing the unique band pattern
specific to the DNA of subjects suffering from the
disorder from step e and the DNA obtained for diagnosis
from step f to determine whether the patterns are the
same or different and to diagnose thereby predisposition
to the disorder if the patterns are the same.

In an embodiment, a disorder associated with the activity
of a specific human GALR3 receptor allele is diagnosed.
In another embodiment, the above-described method may be
used to identify a population of patients having a
specific GALR3 receptor allele, in which population the
disorder may be alleviated by administering to the
subjects a GALR3-selective compound.

This invention provides a method of preparing the
purified GALR3 receptor which comprises: (a) inducing
cells to express GALR3 receptor; (b) recovering the
receptor from the induced cells; and (c) purifying the
receptor so recovered.

This invention provides a method of preparing a purified
GALR3 receptor which comprises: (a) inserting nucleic
acid encoding the GALR3 receptor in a suitable vector;
(b) introducing the resulting vector in a suitable host
cell; (c) placing the resulting cell in suitable

condition permitting the production of the isolated GALR3 receptor; (d) recovering the receptor produced by the resulting cell; and (e) purifying the receptor so recovered.

This invention provides a method of modifying feeding behavior of a subject which comprises administering to the subject an amount of a compound which is a galanin receptor agonist or antagonist effective to increase or decrease the consumption of food by the subject so as to thereby modify feeding behavior of the subject. In one embodiment, the compound is a GALR3 receptor antagonist and the amount is effective to decrease the consumption of food by the subject. In another embodiment the compound is administered in combination with food.

In yet another embodiment the compound is a GALR3 receptor agonist and the amount is effective to increase the consumption of food by the subject. In a still further embodiment, the compound is administered in combination with food. In other embodiments the subject is a vertebrate, a mammal, a human or a canine.

In one embodiment, the compound binds selectively to a GALR3 receptor. In another embodiment, the compound binds to the GALR3 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR1 receptor. In another embodiment, the compound binds to the GALR3 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR2 receptor. In yet another embodiment, the compound binds to the GALR3 receptor with an affinity greater than one hundred-fold higher than the affinity with which the compound binds to a GALR1 receptor. In another embodiment, the compound binds to the GALR3 receptor with an affinity greater than one hundred-fold higher than the affinity with which the

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compound binds to a GALR2 receptor.

This invention provides a method of treating Alzheimer's
disease in a subject which comprises administering to the
5   subject an amount of a compound which is a galanin
receptor antagonist effective to treat the subject's
Alzheimer's disease.   In one embodiment, the galanin
receptor antagonist is a GALR3 receptor antagonist and
the amount of the compound is effective to treat the
10   subject's Alzheimer's disease.

This invention provides a method of producing analgesia
in a subject which comprises administering to the subject
an amount of a compound which is a galanin receptor
15   agonist effective to produce analgesia in the subject.
In another embodiment, the galanin receptor agonist is a
GALR3 receptor agonist and the amount of the compound is
effective to produce analgesia in the subject.

20   This invention provides a method of decreasing
nociception in a subject which comprises administering to
the subject an amount of a compound which is a GALR3
receptor agonist effective to decrease nociception in the
subject.
25
This invention provides a method of treating pain in a
subject which comprises administering to the subject an
amount of a compound which is a GALR3 receptor agonist
effective to treat pain in the subject.
30
This invention provides a method of treating diabetes in
a subject which comprises administering to the subject an
amount of a compound which is a GALR3 receptor antagonist
effective to treat diabetes in the subject.
35
This invention provides a method of decreasing feeding
behavior of a subject which comprises administering a

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compound which is a GALR3 receptor antagonist and a
compound which is a Y5 receptor antagonist, the amount of
such antagonists being effective to decrease the feeding
behavior of the subject.   In an embodiment, the GALR3
5   antagonist and the Y5 antagonist are administered in
combination.   In another embodiment, the GALR3 antagonist
and the Y5 antagonist are administered once.   In another
embodiment, the GALR3 antagonist and the Y5 antagonist
are   administered   separately.   In   still   another
10   embodiment, the GALR3 antagonist and the Y5 antagonist
are administered once.   In another embodiment, the
galanin receptor antagonist is administered for about 1
week to 2 weeks.   In another embodiment, the Y5 receptor
antagonist is administered for about 1 week to 2 weeks.
15

In yet another embodiment, the GALR3 antagonist and the
Y5 antagonist are administered alternately.   In another
embodiment, the GALR3 antagonist and the Y5 antagonist
are   administered   repeatedly.   In   a   still   further
20   embodiment,   the   galanin   receptor   antagonist   is
administered for about 1 week to 2 weeks.   In another
embodiment, the Y5 receptor antagonist is administered
for about 1 week to 2 weeks.   This invention also
provides a method as described above, wherein the
25   compound is administered in a pharmaceutical composition
comprising a sustained release formulation.

This invention will be better understood from the
Experimental Details which follow. However, one skilled
30   in the art will readily appreciate that the specific
methods and results discussed are merely illustrative of
the invention as described more fully in the claims which
follow thereafter.

Experimental Details

Materials and Methods

5   <u>Cloning and Sequencing a novel rat galanin receptor
       fragment</u>

A rat hypothalamus cDNA library in lambda ZAP II ($\approx$2.5 x
10^6 total recombinants; Stratagene, LaJolla, CA) was
screened using overlapping transmembrane (TM)

10   oligonucleotide probes (TM 1, 2, 3, 4, 5, 6 and 7)
derived from the rat GALR2 receptor cDNA. Overlapping
oligomers were labeled with [^{32}P]dATP and [^{3}P]dCTP by
synthesis with the large fragment of DNA polymerase, and
comprised the following sequences:

15

TM1:

(+)strand:

5'TTGTACCCCTATTTTTCGCGCTCATCTTCCTCGTGGGCACCGTGG-3' (SEQ

ID NO: 6);

20   (-)strand:

5'- AGCACCGCCAGCACCAGCGCGTTGCCCACGGTGCCCACGAGGAAG-3' (SEQ

ID NO: 7);

TM2:

25   (+)strand:

5'-TCAGCACCACCAACCTGTTCATCCTCAACCTGGGCGTGGCCGACCTGTGT-3'

(SEQ ID NO: 8);

(-) strand:

5'-GGCCTGGAAAGGCACGCAGCACAGGATGAAACACAGGTCGGCCACGCCCA-3'

30   (SEQ ID NO: 9);

TM3:

(+)strand:

5'-CTGCAAGGCTGTTCATTTCCTCATCTTTCTCACTATGCACGCCAG-3' (SEQ

35   ID NO: 10);

(-) strand:

5'-GGAGACGGCGGCCAGCGTGAAGCTGCTGGCGTGCATAGTGAGAAA-3' (SEQ

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ID NO: 11);

TM4:

(+)strand

5 5'-AACGCGCTGGCCGCCATCGGGCTCATCTGGGGGCTAGCACTGCTC-3' (SEQ
ID NO: 12);

(-)strand

5'-AGTAGCTCAGGTAGGGCCCGGAGAAGAGCAGTGCTAGCCCCCAGA-3' (SEQ
ID NO: 13);

10

TM5:

(+)strand:

5'-AGCCATGGACCTCTGCACCTTCGTCTTTAGCTACCTGCTGCCAGT-3' (SEQ
ID NO: 14);

15 (-)strand:

5'-CGCATAGGTCAGACTGAGGACTAGCACTGGCAGCAGGTAGCTAAA-3' (SEQ
ID NO: 15);

TM6:

20 (+)strand:

5'-GATCATCATCGTGGCGGTGCTTTTCTGCCTCTGTTGGATGCCCCA-3' (SEQ
ID NO: 16);

(-)strand:

5'-CCACACGCAGAGGATAAGCGCGTGGTGGGGCATCCAACAGAGGCA-3' (SEQ
25 ID NO: 17);

TM7:

(+)strand:

5'-GTTGCGCATCCTTTCACACCTAGTTTCCTATGCCAACTCCTGTGT-3' (SEQ
30 ID NO: 18);

(-)strand:

5'-AGACCAGAGCGTAAACGATGGGGTTGACACAGGAGTTGGCATAGGA-3' (SEQ
ID NO: 19).

35

Hybridization of phage lifts was performed at reduced
stringency conditions: 40°C in a solution containing

37.5% formamide, 5x SSC (1X SSC is 0.15M sodium chloride, 0.015M sodium citrate), 1x Denhardt's solution (0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin), and 25 µg/µL sonicated salmon sperm DNA. The

5   filters were washed at 45°C in 0.1x SSC containing 0.1% sodium dodecyl sulfate and exposed at -70°C to Kodak BioMax film in the presence of an intensifying screen. Lambda phage clones hybridizing with the probes were plaque purified and pBluescript recombinant DNAs were

10   excision-rescued from λ Zap II using helper phage Re704, as described by the manufacturer's protocol (Rapid Excision Kit, Stratagene, LaJolla, CA.). Insert size was confirmed by restriction enzyme digest analysis. The cDNA insert was sequenced on both strands by cycle

15   sequencing with AmpliTaq DNA Polymerase, FS (Perkin Elmer) and products run on an automated fluorescent sequencer, the ABI Prism 377 Sequencer (ABI). Nucleotide and peptide sequence analyses were performed using the Wisconsin Package (GCG, Genetics Computer Group, Madison,

20   WI). Sequence analyses indicated that one clone, named rHY35a, contained an open reading frame from the starting MET codon to the middle of a predicted seventh transmembrane domain. Because the high degree of identity of rHY35a to rGALR1 and rGALR2 indicated that it

25   might represent a fragment of a novel galanin receptor (referred to herein as "GALR3"), PCR primers directed to the amino terminus (forward primer) and first extracellular loop (reverse primer) of each of the corresponding receptor cDNA were synthesized having the

30   following sequences:

rGALR1:
(forward primer):
5'-CCTCAGTGAAGGGAATGGGAGCGA-3' (SEQ ID NO: 20);

35   (reverse primer):
5'-GTAGTGTATAAACTTGCAGATGAAGGC-3' (SEQ ID NO: 21);

rGALR2:

(forward primer):

5'-ATGAATGGCTCCGGCAGCCAGGG-3' (SEQ ID NO: 22);

(reverse primer):

5   5'-TTGCAGAGCAGCGAGCCGAACAC-3' (SEQ ID NO: 23); and

rHY35a (i.e., rat GALR3):

(forward primer):

5'-GGCTGACATCCAGAACATTTCGCT-3' (SEQ ID NO: 24);

10   (reverse primer):

5'-CAGATGTACCGTCTTGCACACGAA-3' (SEQ ID NO: 25).

<u>Polymerase Chain Reaction (PCR) of cDNA</u>

Total RNA was prepared from RIN14B cells (ATCC No. CCL

15   89) by a modification of the guanidine thiocyanate method
(Chirgwin et al., 1979). Poly A⁺ RNA was purified with a
FastTrack kit (Invitrogen Corp., San Diego, CA) and
converted to single-stranded cDNA by random priming using
Superscript reverse transcriptase (BRL, Gaithersburg,

20   MD). An aliquot of the first strand cDNA was diluted
(1:50) in a 50 μL PCR reaction mixture containing a
combination of Taq and Pwo DNA polymerases in the buffer
supplied by the manufacturer (for the Expand Long
Template PCR System, Boehringer Mannheim), and 300 nM

25   each of the amino terminus and first extracellular loop
rGALR3 (rHY35a) primers described above. The PCR
amplification reaction was performed under the following
conditions: 30 sec. at 94°C and 1 min. 30 sec. at 68°C
for 40 cycles, with a pre- and post-incubation of 5 min.

30   at 95°C and 2 min. 30 sec. at 68°C, respectively. In
order to control for the amplification of DNA
(potentially carried over during the RNA extraction),
control PCR reactions were run in parallel using RIN14B
RNA prepared as above but without reverse transcriptase,

35   and thus not converted to cDNA. The PCR products were
separated on a 1.0% agarose gel and stained with ethidium
bromide.

Construction and PCR screening of a RIN14B cell line
plasmid library

Total RNA was prepared from RIN14B cells by a
modification of the guanidine thiocyanate method
5   (Chirgwin et al., 1979). Poly A⁺ RNA was purified with a
FastTrack kit (Invitrogen Corp., San Diego, CA). Double
stranded (ds) cDNA was synthesized from 4 µg of poly A⁺
RNA according to Gubler and Hoffman (1983) with minor
modifications. The resulting cDNA was ligated to
10   BstXI/EcoRI adaptors (Invitrogen Corp.) and the excess
adaptors removed by exclusion column chromatography.
High molecular weight fractions of size-selected ds-cDNA
were ligated in pEXJ.BS (an Okayama and Berg expression
vector) and electroporated in E.coli MC 1061 (Gene
15   Pulser, Biorad). A total of 0.9 x 10⁶ independent clones
with an insert mean size of 3.4 kb were generated. The
library was plated on agar plates (Ampicillin selection)
in 216 pools of ~4,000 independent clones. After 18
hours amplification, the bacteria from each pool were
20   scraped, resuspended in 4 mL of LB media, and 1.5 mL
processed for plasmid purification (Qiaprep, Qiagen,
Inc., Chatsworth, CA). Aliquots of each bacterial pool
were stored at -85°C in 20% glycerol.

25   Glycerol stocks (2 µL) of the 216 primary pools for the
RIN14B plasmid library (designated "F") were screened for
rGALR3 by PCR using a forward primer from the third
transmembrane domain of rGALR3 (5'-
CATCTGCTCATCTACCTCACCATG-3' (SEQ ID NO: 26)) and a
30   reverse primer from third intracellular loop of rGALR3
(5'-CATAGGAAACATAGCGTGCGTCCG-3' (SEQ ID NO: 27)). PCR
was performed with the Expand Long Template PCR System,
as described in the preceding section. Two positive
pools, F105 and F212, were subjected to further PCR
35   analyses, using a forward primer to the amino terminus of
rat GALR3 (described above) with a reverse primer from
the third intracellular loop (described above), as well

as vector-anchored PCR (see below). These PCR analyses
indicated that, although these clones were full-length,
they were in the incorrect orientation in the expression
vector (pEXJ.BS). Although these pools were not further
subdivided, the sequence missing from clone rHY35a (i.e.,
from the middle of TM7 through the stop codon) was
determined from the F105 clone, using vector-anchored
PCR, as described below.

Vector-anchored PCR

To determine the orientation and size of the F105 cDNA
insert (including the coding region, 5' untranslated (UT)
and 3' UT regions) PCR was conducted on glycerol stocks
(2µL) using combinations of vector-derived primers and
gene-specific primers. The vector-derived forward primer
sequence was 5'-AAGCTTCTAGAGATCCCTCGACCTC-3' (SEQ ID NO:
28); the reverse primer sequence was 5'-
AGGCGCAGAACTGGTAGGTATGGAA-3' (SEQ ID NO: 29). The
rGALR3-specific forward primer (in the sixth
transmembrane domain) was 5'-GCTCATCCTCTGCTTCTGGTACG-3'
(SEQ ID NO: 30); the reverse primer (in the first
extracellular loop) was 5'-CAGATGTACCGTCTTGCACACGAA-3'
(SEQ ID NO: 31). PCR was performed with the Expand Long
Template PCR System, as described above. The PCR
products were separated on a 1.0% agarose gel and stained
with ethidium bromide.

A 1.2 kb vector-anchored PCR product generated from pool
F105 using the sixth TM forward primer from rGALR3 and
the vector-derived reverse primer was isolated from a 1%
TAE gel using a GENECLEAN III kit (BIO 101, Vista, CA)
and sequenced using AmpliTaq DNA Polymerase, FS (Perkin
Elmer). Sequencing reactions were run on an ABI PRISM
377 DNA Sequencer and analyzed using the Wisconsin
Package (GCG, Genetics Computer Group, Madison, WI). The
sequence information from this vector-anchored PCR
product corresponding to the predicted 3' end of the

novel receptor gene indicated an overlap with rHY35a within the first half of TM7. Downstream of this overlap was new sequence, consistent with the second half of TM7 and the carboxy terminus, including an in-frame stop

5   codon. Based on this newly acquired sequence, a reverse primer, within the 3'UT, was synthesized (also containing a BamHI site at the 5' end, as indicated by the underline): 5'-CGAGGATCCCAACTTTGCCTCTGCTTTTTGGTGG-3' (SEQ ID NO: 32).

10

Construction and PCR screening of a rat hypothalamus plasmid library

Total RNA was prepared from rat hypothalami by a modification of the guanidine thiocyanate method

15   (Chirgwin, 1979). Poly A⁺ RNA was purified using a FastTrack kit (Invitrogen Corp., San Diego, CA). Double stranded (ds) cDNA was synthesized from 6 μg of poly A⁺ RNA according to Gubler and Hoffman (1983) with minor modifications. The resulting cDNA was ligated to

20   BstXI/EcoRI adaptors (Invitrogen Corp.) and the excess adaptors removed by exclusion column chromatography. High molecular weight fractions of size-selected ds-cDNA were ligated in pEXJ.T7 (an Okayama and Berg expression vector modified from pcEXV (Miller & Germain, 1986) to

25   contain BstXI and other additional restriction sites and a T7 promoter (Stratagene) and electroporated in E.coli MC 1061 (Gene Pulser, Biorad). A total of 1.2 x 10⁶ independent clones with a mean insert size of 3.2 kb were generated. The library (designated "K") was plated on

30   agar plates (Ampicillin selection) in 373 primary pools of ~3,200 independent clones. After 18 hours amplification, the bacteria from each pool were scraped, resuspended in 4 mL of LB media and 0.75 mL processed for plasmid purification (QIAwell-96 ultra, Qiagen, Inc.,

35   Chatsworth, CA). Aliquots of each bacterial pool were stored at -85°C in 20% glycerol.

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To screen the library for galanin binding, COS-7 cells were plated in slide chambers (Lab-Tek) in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% calf serum, 100 U/mL of penicillin, 100 ug/mL streptomycin, 2 mM L-glutamine (DMEM-C) and grown at 37°C in a humidified 5% CO_2 atmosphere for 24 hours before transfection. Cells were transfected with miniprep DNA prepared from the primary pools (~3,200 cfu/pool) of the rat hypothalamus cDNA library ("K" library) using a modification of the DEAE-dextran method (Warden & Thorne, 1968). Pools containing GALR1 and GALR2 were identified by PCR prior to screening. The galanin binding assay was carried out after 48 hours. Cells were rinsed twice with phosphate-buffered saline (PBS) then incubated with 2 nM ^{125}I-porcine galanin (NEN; specific activity ~2200 Ci/mmol) in 20mM HEPES-NaOH, pH 7.4, containing 1.26 mM $CaCl_2$, 0.81 mM $MgSO_4$, 0.44 mM KH_2PO_4, 5.4 mM KCl, 10 mM NaCl, 0.1% BSA, and 0.1% bacitracin for one hour at room temperature. After rinsing and fixation in 2.5% glutaraldehyde, slides were rinsed in PBS, air-dried, and dipped in photoemulsion (Kodak, NTB-2). After a 4 day exposure slides were developed in Kodak D19 developer, fixed, and coverslipped (Aqua-Mount, Lerner Laboratories), then inspected for positive cells by brightfield microscopy (Leitz Laborlux, 25X magnification).

PCR screening of the rat hypothalamus cDNA library
Glycerol stocks of the primary pools were combined into 40 superpools of 10 primary pools and screened for rGALR3 by PCR using the same primers as described for the screening of the RIN14B plasmid library (see above). Primary pools from positive superpools (#3 and #17) were inspected for galanin binding using the photoemulsion binding assay described above and screened by PCR. The slide corresponding to pool K163 exhibited positive galanin binding. Pool K163 was then subjected to PCR with internal rGALR3 primers (TM3 forward primer and

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third intracellular loop reverse primer; described
above), full-length primers (forward primer to the amino
terminus, at the starting MET, and reverse primer to the
3' UT (containing a Bam HI site as above)) and with the
5   vector and gene-specific primers (preceding section).
These PCR analyses indicated that the primary pool K163
contained a full-length coding region for rGALR3 in the
correct orientation in the expression vector, pEXJ.T7.
Pool K163 was further analyzed by PCR and shown to
10   contain GALR3 but not GALR1 nor GALR2, indicating that a
novel galanin receptor cDNA was present in the pool and
responsible for the galanin binding. The PCR primers
used to confirm the absence of GALR1 and GALR2 in the
pool are described below:

15

rGALR1:
Forward primer, KS-1311:
5'-CCTCAGTGAAGGGAATGGGAGCGA (SEQ ID NO: 33);
Reverse primer, KS-1447:
20   5'-CTTGCTTGTACGCCTTCCGGAAGT (SEQ ID NO: 34);

Human GALR1:
Forward primer, KS-1177:
5'-TGGGCAACAGCCTAGTGATCACCG-3' (SEQ ID NO: 35);
25   Reverse primer, KS-1178:
5'-CTGCTCCCAGCAGAAGGTCTGGTT-3' (SEQ ID NO: 36);

rGALR2:
Forward primer, KS-1543:
30   5'-ATGAATGGCTCCGGCAGCCAGGG-3' (SEQ ID NO: 37);
Reverse primer, KS-1499:
5'-TTGGAGACCAGAGCGTAAACGATGG-3' (SEQ ID NO: 38).

The primary pool K163 was further subdivided and screened
35   by PCR. One positive subpool, 163-30, was subdivided
into 15 pools of 150 clones and 15 pools of 500 clones
and plated on agar plates (ampicillin selection).

Colonies were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH), denatured in 0.4 N NaOH, 1.5 M NaCl, renatured in 1M Tris, 1.5 M NaCl, and UV cross-linked. Filters were hybridized overnight at

5   40°C in a buffer containing 50 % formamide, 5X SSC, 7 mM TRIS, 1X Denhardt's solution and 25 μg/mL salmon sperm DNA (Sigma Chemical Co.) and 10^6 cpm/ml of overlapping 45-mer oligonucleotide probes, filled-in using [α-^{32}P]dCTP and [α-^{32}P]dATP (800Ci/mmol, NEN) and Klenow fragment of

10   DNA polymerase (Boehringer Mannheim). The following probe sequence is directed to the amino terminus of rGALR3:

from the sense strand:

15   5'-AGATGGCTGACATCCAGAACATTTCGCTGGACAGCCCAGGGAGCG-3' (SEQ ID NO: 39);

from the antisense strand:
5'-ATCACAGGCACTGCCACAGCCCCTACGCTCCCTGGGCTGTCCAGCG-3' (SEQ

20   ID NO: 40).

Filters were washed 2 x 15 minutes at room temperature in 2X SSC, 0.1% SDS, 2 x 15 minutes at 50°C in 0.1X SSC, 0.1% SDS, and exposed to BioMax MS X-ray film (Kodak)

25   with corresponding Kodak intensifying screens for 6 hours. One positive colony, 163-30-17, was amplified overnight separately in 100 mL LB media and in 100 mL TB media and processed for plasmid purification using a standard alkaline lysis miniprep procedure followed by a

30   PEG precipitation. Clone K163-30-17 was sequenced on both strands using AmpliTaq DNA Polymerase, FS (Perkin Elmer). Sequencing reactions were run on an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI).

35   Clone K163-30-17 was given the designation K1086 and deposited with the ATCC (Accession No. 97747).

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Expression in COS-7 cells for whole cell-slide binding

To test the ability of K163-30-17 to confer galanin binding, COS-7 cells were plated in slide chambers (Lab-Tek) in Dulbecco's modified Eagle medium (DMEM)
5   supplemented with 10% calf serum, 100U/mL of penicillin, 100 μg/mL streptomycin, 2mM L-glutamine (DMEM-c) and grown at 37°C in a humidified 5% CO_2 atmosphere for 24 hours before transfection. Cells were transfected with 1 μg of miniprep DNA from K163-30-17 or vector control
10   using a modification of the DEAE-dextran method (Warden and Thorne, 1968). 48 hours after transfection, cells were rinsed with phosphate-buffered saline (PBS) then incubated with 2 nM ^{125}I-porcine galanin (NEN; specific activity ~2200 Ci/mmol) in 20mM HEPES-NaOH, pH 7.4,
15   containing 1.26 mM $CaCl_2$, 0.81 mM $MgSO_4$, 0.44 mM KH_2PO_4, 5.4 mM KCl, 10 mM NaCl, 0.1% BSA, and 0.1% bacitracin for one hour at room temperature. After rinsing and fixation in 2.5% glutaraldehyde, binding of ^{125}I-galanin to cells on the slide was detected by autoradiography using BioMax
20   MS film (Kodak) and an intensifying screen (Kodak). The signal from K163-30-17 transfected cells was compared with the signal from control vector transfected cells.

Cloning and Sequencing a novel human galanin receptor
25   fragment

A human placenta genomic library in λ dash II (≈1.5 x 10^6 total recombinants; Stratagene, LaJolla, CA) was screened using the same set of overlapping oligonucleotide probes to TM regions 1-7 of rat GALR2 and under the same
30   hybridization and wash conditions as described for screening the rat hypothalamus cDNA library (supra). Lambda phage clones hybridizing with the probe were plaque purified and DNA was prepared for Southern blot analysis (Southern, 1975; Sambrook et al., 1989).
35

One phage clone, plc21a, contained a 2.7 kb KpnI/EcoRI fragment which hybridized with the rat GALR2 TM2

oligonucleotide probe and was subsequently subcloned into
a pUC vector. Nucleotide sequence analysis was
accomplished by sequencing both strands using cycle
sequencing with AmpliTaq DNA Polymerase, FS (Perkin

5   Elmer) and products run on the automated fluorescent
sequencer, the ABI Prism 377 Sequencer (ABI), and
sequence analyses were performed using the Wisconsin
Package (GCG, Genetics Computer Group, Madison, WI). DNA
sequence analysis indicated greatest homology to the rat

10   and human GALR1 and GALR2 genes. This clone was a
partial intron-containing gene fragment, encoding the
starting MET through to an intron in the second
intracellular loop (i.e., TM 3/4 loop).

15   Isolation of the full-length human GALR3 receptor gene
Sequence analyses of the cloned human genomic fragment
indicated the presence of a open reading frame from the
starting MET codon down to a predicted intron in the
second intracellular loop, with a nucleotide identity of

20   88% (93% amino acid identity) with the rat GALR3 receptor
described above (thus establishing this human genomic
clone to be the human homologue of rat GALR3). Although
this human genomic fragment was not full-length and
contained an intron downstream of TM3, it is anticipated

25   that a molecular biologist skilled in the art may isolate
the full-length, intronless version of the human GALR3
receptor gene using standard molecular biology techniques
and approaches such as those briefly described below:

30   Approach #1: Using PCR to screen commercial human cDNA
phage libraries and in-house human cDNA plasmid libraries
with primers to the human GALR3 sequence (forward primer
in amino terminus, 5'-ATGGCTGATGCCCAGAACATTTCAC-3' (SEQ
ID NO: 41), and reverse primer in first extracellular

35   loop, 5'-AGCCAGGCATCCAGCGTGTAGAT-3' (SEQ ID NO: 42), we
have identified two commercial libraries and two
proprietary plasmid libraries that contain at least part

of the human GALR3 gene, as follows:

human fetal brain cDNA lambda ZAPII library (Stratagene);
human testis cDNA lambda ZAPII library (Stratagene);
5   human hypothalamus cDNA plasmid library (proprietary)--3
superpools identified; and
human hippocampus cDNA plasmid library (proprietary)--3
superpools identified.

10   One may determine whether these libraries contain full-
length human GALR3 by: (1) obtaining a purified clone
from the lambda libraries by plaque-purification and then
conducting hybridization screening using probes derived
from rat GALR3 under reduced stringency, using standard
15   protocols and/or (2) using PCR to determine which pool of
the human plasmid library superpools contain the gene and
then conducting vector-anchor PCR (as described in this
patent) to determine if these cDNAs are full-length. One
problem which may arise with vector-anchored PCR is a
20   false-positive result, in which the PCR product size is
consistent with a full-length clone but the product
actually contains an intron in the second intracellular
loop. In this case, sequencing of this product would
identify whether this product contains the intron or is
25   intronless and full-length (also see Approach #2 below).

Approach #2: We have also determined that the phage
clone containing MET thru the intron in the second
intracellular loop (i.e., TM3/4 loop), plc21a (see
30   above), also contains at least part of the 3' end of the
gene, by using hybridization at reduced stringency with
a probe to the third extracellular loop (TM 6/7) derived
from the rat GALR3 sequence:

35   5'-ACGGTCGCTTCGCCTTCAGCCCGGCCACCTACGCCTGTCGCCTGG-3' (SEQ
ID NO: 43).

Standard molecular biology techniques may be used to
subclone either the entire intron-containing full-length
human GALR3 (with confirmation that it contains an in-
frame stop codon) or subclone the part of the gene from
5   the intron in the second intracellular loop through the
stop codon. This approach would permit one to utilize
sequence around the termination codon to design a primer
which can be used with the primer around the starting
MET, to generate the full-length intronless human GALR3
10   gene, using human cDNA as the target template.
Alternatively, one may use restriction enzymes to remove
the intron and some adjacent coding region from the
.intron-containing human GALR3 gene, and then replace the
removed coding region by inserting a restriction enzyme-
15   digested PCR fragment amplified from a tissue shown to
express the intronless form of the receptor.

Approach #3: As yet another alternative method, one could
utilize 3' RACE to generate a PCR product from human cDNA
20   expressing human GALR3 (e.g., human brain), using a
forward primer derived from known sequence between the
starting MET thru the second intracellular loop (from the
fragment already isolated). Such a PCR product could
then be sequenced to confirm that it contains the rest of
25   the coding region (without an intron), and then attached
to the 5' end of the molecule, using an overlapping
restriction site, or alternatively, its sequence could be
used to design a reverse primer in the predicted 3' UT
region to generate the full-length, intronless human
30   GALR3 receptor gene with use of the primer at the
starting MET codon and using human cDNA as target
template.

To this end, we have also determined that the phage clone
35   containing MET through the intron in the second
intracellular loop (i.e. TM 3/4 loop), plc21a (see
above), also contains at least part of the 3' end of the

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gene, by using hybridization at reduced stringency with probes either to the third extracellular loop (TM 6/7) or to TM 4, derived from the rat GALR3 sequence:

5   5'-ACGGTCGCTTCGCCTTCAGCCCGGCCACCTACGCCTGTCGCCTGG-3' (SEQ ID NO: 44)
        5'-GCGCAACGCGCGCGCCGCCGTGGGGCTCGTGTGGCTGCTGGCGGC-3' (SEQ ID NO: 45).

10   Another clone, plc14a, which was essentially the same as plc21a (i.e. possessed the identical restriction map and hybridizing bands as plc21a), was further utilized by subcloning a 1.4kb KpnI fragment which similarly hybridized to the above probes. Since the phage clone,
15   plc14a, also hybridized with a TM2/3 loop probe under high stringency, derived from sequence data of human GALR3 5' fragment (plc21a, see above),

        5"-ATCTACACGCTGGATGCCTGGCTCTTTGGGGCCCTCGTCTGCAAG-3' (SEQ
20   ID NO: 46),

        this 3' fragment (e.g. plc14a) presumably corresponds to the 3' end of human GALR3 and is molecularly linked to the 5' fragment (e.g. plc21a 2.7kb KpnI/EcoRI clone);
25   however, an intron of unknown size separates the coding region, which is defined on the 5' (2.7kb KpnI/EcoRI plc21a fragment) and 3' (1.4kb KpnIplc14a fragment) genomic pieces. Nucleotide sequence analysis was conducted on the 1.4 kb KpnI plc14a fragment, as
30   described above, and indicated greatest homology to the rat and human GALR1 and GALR2 genes.

        To obtain sequence information from the region defined by the intersection of these to exons as well as to prove
35   that the 5' and 3' fragments, putatively representing the entire full-length coding region of human GALR3, are molecularly linked, we used a forward oligonucleotide

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primer located on the 5' fragment (within 2/3 loop)

5'-ATCTACACGCTGGATGCCCTGGCT-3' (SEQ ID NO: 47) and
a reverse oligonucleotide primer located on the 3'
5   fragment (within the predicted 4/5 loop),

5'-CGTAGCGCACGGTGCCGTAGTA-3' (SEQ ID NO: 48),

to amplify human brain and liver cDNA (corresponding to
10   5 ng of poly⁺ RNA). The predicted ≈250 nts. PCR products
were sequenced and demonstrated that: (1) the sequences
were identical between brain and liver cDNA, (2) the 5'
and 3' genomic fragments are linked and represent the 5'
and 3' fragments of the human GALR3 gene, and (3) the
15   sequence obtained defined the junction of the exon
containing the starting MET through the 3/4 loop (e.g.,
housed on the 2.7 kb KpnI/EcoRI plc21a subclone) and the
exon containing the 3/4 loop through the predicted STOP
codon (e.g. housed on the 1.4 kb KpnI plc14a subclone).
20   The sequence of this junction demonstrated the presence
of a KpnI site, which was utilized in the construction of
the full-length gene.

The construction of the full-length human GALR3 gene
25   first involved the generation of the 5' end of the gene
using PCR to synthetically create a KpnI site at the 3'
end of the PCR product. To this end, we designed a
forward oligonucleotide primer located at the starting
MET of the 5' fragment and added a consensus Kozak
30   sequence as well as a BamHI site to be used for
subcloning:

5'-GATGGATCCGCCACCATGGCTGATGCCCAGAACATTTCAC-3'   (SEQ ID
NO: 49),
35

and a reverse oligonucleotide primer, within the 3/4
loop, containing a KpnI site that generated the joint

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between the 5' and 3' KpnI fragment:

5'-GCAGGTACCTGTCCACGGAGACAGCAGC-3' (SEQ ID NO: 50).

5   The addition of the KpnI site enabled the attachment of
the 3' KpnI fragment but preserved the sequence which was
identified from human brain and liver cDNAs.

The forward and reverse primers were used to amplify the
10   2.7kb KpnI/EcoRI5' genomic-containing plasmid (plc21a)
using PCR, as described in a previous section but
utilizing Expand High Fidelity PCR System (Boehringer
,Manniheim). The PCR product was isolated from a low
melting gel, purified by phenol extraction, digested with
15   BamHI and KpnI and purified further by phenol extraction.
This BamHI/KpnI PCR product was subcloned into
BamHI/KpnI-digested expression vector, pEXJ, and
sequenced. The sequence of the PCR product was identical
to that determined for the original genomic fragment.
20   The subclone was then digested with KpnI, treated with
calf intestinal alkaline phosphatase, and ligated with
the 1.4 KpnI 3' genomic fragment. Correct orientation
was determined by both restriction mapping and
sequencing. Therefore, the full-length human GALR3
25   construct contained = 1.7kb genomic insert, containing
1107 bp of coding region and = 600 bp of 3' non-coding
region.

Northern Blots

30   Rat multiple tissue northern blots (rat MTN blot,
Clontech, Palo Alto, CA), containing 2 μg poly A⁺ RNA, or
northern blots containing 5 μg poly' RNA, either purchased
from Clontech or purified from various rat peripheral
tissues and brain regions, respectively, were similarly
35   hybridized at high stringency with a probe directed to
the amino-terminus of rGalR3 (SEQ ID NO 39 and 40),
according to the manufacturer's specifications. Probe

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was labeled as previously described (supra), using Klenow fragment of DNA polymerase, except $[\alpha-{}^{32}P]dCTP$ and $[\alpha-{}^{32}P]dATP$ (3000Ci/mmol, NEN) were used. Northern blots were reprobed with a randomly-primed β-actin probe to
5   assess quantities of mRNA present in each lane.

Human brain multiple tissue northern blots (MTN brain blots II and III, Clontech, Palo Alto, CA) and human peripheral MTN blot (Clontech, Palo Alto, CA) carrying
10 mRNA (2 μg) purified from various human brain areas and peripheral tissues, respectively, were hybridized at high stringency with overlapping probes directed to the amino-terminus of hGALR3
    5' GATGGCTGATGCCCAGAACATTTCACTGGACAGCCCAGGGAGTGT 3'
15 (SEQ ID NO. 51) and
    5' GACCACAGGCACTGCCACGGCCCCCACACTCCCTGGGCTGTCCAG 3'
    (SEQ ID NO. 52), according to the manufacturer's specifications.

20   <u>RT-PCR analyses of GALR3 mRNA</u>
    Tissues were homogenized and total RNA extracted using the guanidine isothiocyanate/CsCl cushion method. RNA was then treated with DNase to remove any contaminating genomic DNA and poly A^+-selected using FastTrack kit
25 (Invitrogen), according to manufacturer's specifications. cDNA was prepared from mRNA with random hexanucleotide primers using reverse transcriptase Superscript II (BRL, Gaithersburg, MD). First strand cDNA (corresponding to $\approx$5 ng of poly A^+ RNA) was amplified in a 50 μL PCR
30 reaction mixture with 300 nM of forward (directed to the amino-terminus: SEQ ID NO. 24) and reverse (directed to the third intracellular loop: SEQ ID NO. 27) primers, using the thermal cycling program and conditions described above.

35

The PCR products were run on a 1.5% agarose gel and transferred to charged nylon membranes (Zetaprobe GT,

BioRad), and analyzed as Southern blots. GALR3 primers were screened for the absence of cross-reactivity with the other galanin receptors. Filters were hybridized with a radiolabeled probe directed to the first
5 intracellular loop,
5'-TGCAGCCTGGCCCAAGTGCCTGGCAGGAGCCAAGCAGTACCACAG-3' (Seq. I.D. No. 53), and washed under high stringency. Labeled PCR products were visualized on X-ray film. Similar PCR and Southern blot analyses were conducted with primers
10 and probes directed to the housekeeping gene, glyceraldehyde phosphate dehydrogenase (G3PDH; Clontech, Palo Alto, CA), to normalize the amount of cDNA used from the different tissues.

15 Production of Recombinant Baculovirus
The coding region of GALR3 may be subcloned into pBlueBacIII into existing restriction sites, or sites engineered into sequences 5' and 3' to the coding region of GALR3, for example, a 5' EcoRI site and a 3' EcoRI
20 site. To generate baculovirus, 0.5 μg of viral DNA (BaculoGold) and 3 μg of GALR3 construct may be co-transfected into 2 x 10^6 *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as outlined in by Pharmingen (in "Baculovirus Expression
25 Vector System: Procedures and Methods Manual"). The cells then are incubated for 5 days at 27°C.

The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus
30 plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks are as described in Pharmingen's manual.

Cell Culture
35 COS-7 cells are grown on 150 mm plates in DMEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL

penicillin/100 μg/mL streptomycin) at 37°C, 5% CO_2. Stock plates of COS-7 cells are trypsinized and split 1:6 every 3-4 days. Human embryonic kidney 293 cells are grown on 150 mm plates in D-MEM with supplements (minimal
5   essential medium) with Hanks' salts and supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 μg/mL streptomycin) at 37°C, 5% CO_2. Stock plates of 293 cells are trypsinized and split 1:6 every 3-4 days. Mouse
10 fibroblast LM(tk-) cells are grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 μg/mL streptomycin) at 37°C, 5% CO_2. Stock plates of LM(tk-) cells are trypsinized and split 1:10
15 every 3-4 days.

LM(tk-) cells stably transfected with the GALR3 receptor may be routinely converted from an adherent monolayer to a viable suspension. Adherent cells are harvested with
20 trypsin at the point of confluence, resuspended in a minimal volume of complete DMEM for a cell count, and further diluted to a concentration of 10^6 cells/mL in suspension media (10% bovine calf serum, 10% 10X Medium 199 (Gibco), 9 mM $NaHCO_3$, 25 mM glucose, 2 mM L-glutamine,
25 100 units/mL penicillin/100 μg/mL streptomycin, and 0.05% methyl cellulose). Cell suspensions are maintained in a shaking incubator at 37°C, 5% CO_2 for 24 hours. Membranes harvested from cells grown in this manner may be stored as large, uniform batches in liquid nitrogen.
30 Alternatively, cells may be returned to adherent cell culture in complete DMEM by distribution into 96-well microtiter plates coated with poly-D-lysine (0.01 mg/mL) followed by incubation at 37°C, 5% CO_2 for 24 hours. Cells prepared in this manner generally yield a robust
35 and reliable response in cAMP radio-immunoassays as further described hereinbelow.

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Mouse embryonic fibroblast NIH-3T3 cells are grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 μg/mL streptomycin) at 37°C, 5% CO2. Stock plates of NIH-3T3 cells are trypsinized and split 1:15 every 3-4 days. Chinese hamster ovary (CHO) cells were grown on 150 mm plates in HAM's F-12 medium with supplements (10% bovine calf serum, 4 mM L-glutamine and 100 units/mL penicillin/100 ug/ml streptomycin) at 37°C, 5% CO2. Stock plates of CHO cells were trypsinized and split 1:8 every 3-4 days.

Sf9 and Sf21 cells are grown in monolayers on 150 mm tissue culture dishes in TMN-FH media supplemented with 10% fetal calf serum, at 27°C, no CO_2. High Five insect cells are grown on 150 mm tissue culture dishes in Ex-Cell 400™ medium supplemented with L-Glutamine, also at 27°C, no CO_2.

Transfection

All receptor subtypes studied may be transiently transfected into COS-7 cells by the DEAE-dextran method, using 1 μg of DNA /10^6 cells (Cullen, 1987). In addition, Schneider 2 Drosophila cells may be cotransfected with vectors containing the receptor gene, under control of a promoter which is active in insect cells, and a selectable resistance gene, eg., the G418 resistant neomycin gene, for expression of the galanin receptor.

Stable Transfection

The GALR3 receptor may be co-transfected with a G-418 resistant gene into the human embryonic kidney 293 cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells are selected with G-418. GALR3 receptors may be similarly transfected into mouse fibroblast LM(tk-) cells, Chinese hamster ovary (CHO) cells and NIH-3T3 cells, or other suitable host cells.

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GALR1 receptors were expressed in cells using methods well-known in the art.

Radioligand binding assays

5   Transfected cells from culture flasks are scraped into of 20 mM Tris-HCl, 5mM EDTA, pH 7.5, and lysed by sonication. The cell lysates are centrifuged at 1000 rpm for 5 min. at 4°C, and the supernatant centrifuged at 30,000 x g for 20 min. at 4°C. The pellet is suspended

10   in binding buffer (50mM Tris-HCl, 5mM $MgSO_4$, 1mM EDTA at pH 7.5 supplemented with 0.1% BSA, $2\mu g/ml$ aprotinin, $0.5mg/ml$ leupeptin, and $10\mu g/ml$ phosphoramidon). Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10% of the added

15   radioligand, are added to 96-well polpropylene microtiter plates containing ^{125}I-labeled peptide, non-labeled peptides and binding buffer to a final volume of 250 μl. In equilibrium saturation binding assays membrane preparations may be incubated in the presence of

20   increasing concentrations (e.g., 0.1 nM to 4 nM) of [^{125}I]porcine galanin (specific activity about 2200 Ci/mmol). The binding affinities of the different galanin analogs may be determined in equilibrium competition binding assays, using 0.1-0.5 nM [^{125}I]porcine

25   galanin in the presence of e.g., twelve different concentrations of the displacing ligands. Binding reaction mixtures are incubated for 1 hr at 30°C, and the reaction stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell

30   harvester. Radioactivity may be measured by scintillation counting and the data analyzed by a computerized non-linear regression program. Non-specific binding may be defined as the amount of radioactivity remaining after incubation of membrane protein in the

35   presence of 100nM of unlabeled porcine galanin. Protein concentration may be measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a

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standard.   Such competitive binding assays are well-known in the art, and may also include the use of non-hydrolyzable analogues of GTP, which may reduce the binding of agonists to the GALR3 receptors of the present invention.

The binding assays used to generate the data shown in Table 4 were conducted as described above, with certain modifications. Assays were conducted at room temperature for 120 minutes, and leupeptin, aprotonin and phosphoramidon were omitted from the rat GALR3 assay, while bacitracin was added to 0.1%.   In addition, nonspecific binding was defined in the presence of 1 μM porcine galanin.

Functional Assays
Cyclic AMP (cAMP) formation
The receptor-mediated inhibition of cyclic AMP (cAMP) formation may be assayed in LM(tk-) cells expressing the galanin receptors.   Cells are plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5mM theophylline, 2μg/ml aprotinin, 0.5mg/ml leupeptin, and 10μg/ml phosphoramidon for 20 min at 37°C, in 5% CO_2. Galanin or the test compounds are added and incubated for an additional 10 min at 37°C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl. The plates are stored at 4°C for 15 min, and the cAMP content in the stopping solution measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software.

Arachidonic Acid Release
CHO cells stably transfected with the rat GALR3 receptor are seeded into 96 well plates and grown for 3 days in HAM's F-12 with supplements. ^{3}H-arachidonic acid (specific activity = 0.75 uCi/ml) is delivered as a 100

uL aliquot to each well and samples were incubated at 37°
C, 5% CO_2 for 18 hours. The labeled cells are washed three
times with 200 uL HAM's F-12. The wells are then filled
with medium (200 uL) and the assay is initiated with the
addition of peptides or buffer (22 uL). Cells are
incubated for 30 min at 37°C, 5% CO2. Supernatants are
transferred to a microtiter plate and evaporated to
dryness at 75°C in a vacuum oven. Samples are then
dissolved and resuspended in 25 uL distilled water.
Scintillant (300 uL) is added to each well and samples
are counted for ^3H in a Trilux plate reader. Data are
analyzed using nonlinear regression and statistical
techniques available in the GraphPAD Prism package (San
Diego, CA).

Intracellular calcium mobilization
The intracellular free calcium concentration may be
measured by microspectroflourometry using the fluorescent
indicator dye Fura-2/AM (Bush et al. 1991). Stably
transfected cells are seeded onto a 35 mm culture dish
containing a glass coverslip insert. Cells are washed
with HBS and loaded with 100 μL of Fura-2/AM (10 μM) for
20 to 40 min. After washing with HBS to remove the Fura-
2/AM solution, cells are equilibrated in HBS for 10 to 20
min. Cells are then visualized under the 40X objective
of a Leitz Fluovert FS microscope and fluorescence
emission is determined at 510 nM with excitation
wavelengths alternating between 340 nM and 380 nM. Raw
fluorescence data are converted to calcium concentrations
using standard calcium concentration curves and software
analysis techniques.

Phosphoinositide metabolism
LM(tk-) cells stably expressing the rat GALR3 receptor
cDNA are plated in 96-well plates and grown to
confluence. The day before the assay the growth medium
is changed to 100 μl of medium containing 1% serum and

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0.5 μCi [³H]myo-inositol, and the plates are incubated overnight in a CO_2 incubator (5% CO_2 at 37⁰C). Alternatively, arachidonic acid release may be measured if [³H]arachidonic acid is substituted for the [H]myo-inositol. Immediately before the assay, the medium is removed and replaced by 200 μL of PBS containing 10 mM LiCl, and the cells are equilibrated with the new medium for 20 min. During this interval cells are also equilibrated with the antagonist, added as a 10 μL aliquot of a 20-fold concentrated solution in PBS. The [³H]inositol-phosphates accumulation from inositol phospholipid metabolism may be started by adding 10 μL of a solution containing the agonist. To the first well 10 μL may be added to measure basal accumulation, and 11 different concentrations of agonist are assayed in the following 11 wells of each plate row. All assays are performed in duplicate by repeating the same additions in two consecutive plate rows. The plates are incubated in a CO_2 incubator for 1 hr. The reaction may be terminated by adding 15 μl of 50% v/v trichloroacetic acid (TCA), followed by a 40 min. incubation at 4⁰C. After neutralizing TCA with 40 μl of 1M Tris, the content of the wells may be transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared adding 200 μL of Dowex AG1-X8 suspension (50% v/v, water: resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well is washed 2 times with 200 μL of water, followed by 2 x 200 μL of 5mM sodium tetraborate/60 mM ammonium formate. The [³H]IPs are eluted into empty 96-well plates with 200 μl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells is added to 3 mL of scintillation cocktail, and the radioactivity is determined by liquid scintillation counting.

GTPγS functional assay

Membranes from cells transfected with the GALR3 receptors are suspended in assay buffer (50 mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, pH 7.4) supplemented with 0.1% BSA, 0.1% bacitracin and 10 μM GDP. Membranes are incubated on ice
5 for 20 minutes, transferred to a 96-well Millipore microtiter GF/C filter plate and mixed with GTPγ^{35}S (e.g., 250,000 cpm/sample, specific activity ~1000 Ci/mmol) plus or minus GTPγS (final concentration = 100 μM). Final membrane protein concentration $\approx$ 90 μg/mL. Samples are
10 incubated in the presence or absence of porcine galanin (final concentration = 1 μM) for 30 min. at room temperature, then filtered on a Millipore vacuum manifold and washed three times with cold assay buffer. Samples collected in the filter plate are treated with
15 scintillant and counted for ^{35}S in a Trilux (Wallac) liquid scintillation counter. It is expected that optimal results are obtained when the GALR3 receptor membrane preparation is derived from an appropriately engineered heterologous expression system, i.e., an
20 expression system resulting in high levels of expression of the GALR3 receptor and/or expressing G-proteins having high turnover rates (for the exchange of GDP for GTP). GTPγS assays are well-known in the art, and it is expected that variations on the method described above,
25 such as are described by e.g., Tian et al. (1994) or Lazareno and Birdsall (1993), may be used by one of ordinary skill in the art.

The binding and functional assays described herein may
30 also be performed using GALR1 and GALR2 receptors. The GALR1 receptors are well-known in the art and may be prepared and transfected into cells (transiently and stably) using standard methods. Applicants have isolated and cloned the rat and human GALR2 receptors, and have
35 deposited several plasmids expressing GALR2 receptors, as well as cell lines stably expressing the rat GALR2 receptor. Plasmids expressing GALR2 receptors may be

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transiently or stably transfected into cell using methods well-known in the art, examples of which are provided herein. The rat GALR2 receptor may be expressed using plasmid K985 (ATCC Accession No. 97426, deposited January
5   24, 1996), or using plasmid K1045 (ATCC Accession No. 97778, deposited October 30, 1996). Plasmid K1045 comprises an intronless construct encoding the rat GALR2 receptor. Cell lines stably expressing the rat GALR2 receptor have also been prepared, for example, the LM(tk-
10   ) cell lines L-rGALR2-8 (ATCC Accession No. CRL-12074, deposited March 28, 1996) and L-rGALR2I-4 (ATCC Accession No. CRL-12223, deposited October 30, 1996). L-rGALR2I-4 comprises an intronless construct expressing the rat GALR2 receptor. The CHO cell line C-rGalR2-79 (ATCC
15   Accession No. CRL-12262, deposited January 15, 1997) also stably expresses the rat GALR2 receptor. The human GALR2 receptor may be expressed using plasmid BO29 (ATCC Accession No. 97735, deposited September 25, 1996) or plasmid BO39 (ATCC Accession No. 97851, deposited January
20   15, 1997). Plasmid BO39 comprises an intronless construct encoding the human GALR2 receptor.

The plasmids and cell lines described above were deposited with the American Type Culture Collection
25   (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

30   It is to be understood that the cell lines described herein are merely illustrative of the methods used to evaluate the binding and function of the galanin receptors of the present invention, and that other suitable cells may be used in the assays described
35   herein.

Methods for recording currents in Xenopus oocytes

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Female *Xenopus laevis* (Xenopus-1, Ann Arbor, MI) are anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical Corp.) and a portion of ovary is removed using aseptic technique (Quick and Lester, 1994).

5   Oocytes are defolliculated using 2 mg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) in a solution containing 87.5 mM NaCl, 2 mM KCl, 2 mM $MgCl_2$ and 5 mM HEPES, pH 7.5. Oocytes are injected (Nanoject, Drummond Scientific, Broomall, PA) with 50 nL of rat

10   GalR3 mRNA. Other oocytes are injected with a mixture of GalR3 mRNA and mRNA encoding the genes for G-protein-activated inward rectifiers (GIRK1 and GIRK4). Genes encoding GIRK1 and GIRK4 are obtained using conventional PCR-based cloning techniques based on published sequences

15   (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995). RNAs are prepared from separate DNA plasmids containing the complete coding regions of GalR3, GIRK1 and GIRK4. Plasmids are linearized and transcribed using the T7 polymerase ("Message Machine", Ambion).

20   Alternatively, mRNA may be translated from a template generated by PCR, incorporating a T7 promoter and a poly A^+ tail. After injection of mRNA, oocytes are incubated at 16° on a rotating platform for 3-8 days. Dual electrode voltage clamp ("GeneClamp", Axon Instruments

25   Inc., Foster City, CA) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-3 Mohms. Unless otherwise specified, oocytes are voltage clamped at a holding potential of -80 mV. During recordings, oocytes are bathed in continuously flowing (2-5 ml/min)

30   medium containing 96 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 ("ND96"), or, in the case of oocytes expressing GIRK1 and GIRK4, elevated K^+ containing 96 mM KCl, 2 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 ("hK"). Drugs are applied by switching

35   from a series of gravity fed perfusion lines.

Heterologous expression of GPCRs in *Xenopus* oocytes has

been widely used to determine the identity of signaling
pathways activated by agonist stimulation (Gundersen et
al., 1983; Takahashi et al., 1987). Activation of the
phospholipase C (PLC) pathway is assayed by applying 1 μM
galanin in ND96 solution to oocytes previously injected
with mRNA for the GalR3 receptor and observing inward
currents at a holding potential of -80 mV. The
appearance of currents that reverse at -25 mV and display
other properties of the Ca^{++}-activated Cl- channel is
indicative of GalR3 receptor-activation of PLC and
release of IP3 and intracellular Ca^{++}. Subsequently,
measurement of inwardly rectifying K^+ channel (GIRK)
activity is monitored in oocytes that have been co-
injected with mRNAs encoding GALR3, GIRK1 and GIRK4.
These two GIRK gene products co-assemble to form a G-
protein activated potassium channel known to be activated
(i.e., stimulated) by a number of GPCRs that couple to G_i
or G_o (Kubo et al., 1993; Dascal et al., 1993). Oocytes
expressing GalR3 plus the two GIRK subunits are tested
for galanin responsivity using 1 μM galanin and measuring
K^+ currents in elevated K^+ solution (hK). Activation of
inwardly rectifying currents that are sensitive to 300 μM
Ba^{++} signifies GALR3 coupling to a G_i or G_o pathway in the
oocytes.

Oocytes were isolated as described above, except that 3
mg/mL collagenase was used to defolliculate the oocytes.
Genes encoding G-protein inwardly rectifying K^+ channels
1 and 4 (GIRK1 and GIRK4) were obtained by PCR using the
published sequences (Kubo et al., 1993; Dascal et al.,
1993; Krapivinsky et al., 1995b) to derive appropriate 5'
and 3' primers. Human heart cDNA was used as template
together with the primers
5'-CGCGGATCCATTATGTCTGCACTCCGAAGGAAATTTG-3' (SEQ ID NO.
54) and
5'-CGCGAATTCTTATGTGAAGCGATCAGAGTTCATTTTTC -3' (SEQ ID NO.
55) for GIRK1 and

5'-GCGGGATCCGCTATGGCTGGTGATTCTAGGAATG-3' (SEQ ID NO. 56)
and

5'- CCGGAATTCCCCTCACACCGAGCCCCTGG-3' (SEQ ID NO. 57) for
GIRK4. In each primer pair, the upstream primer contained
a BamHI site and the downstream primer contained an EcoRI
site to facilitate cloning of the PCR product into
pcDNA1-Amp (Invitrogen). The transcription template for
hGalR3 was obtained similarly by PCR using the cloned
cDNA in combination with primers

5'-CCAAGCTTCTAATACGACTCACTATAGGGCCACCATGGCTGATGCCCAGA-3'
(SEQ ID NO. 58) and

5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCAGGG
TTTATTCCGGTCCTCG-3' (SEQ ID NO. 59). Alternatively, the
complete coding region of hGalR3 is subcloned into the
high-efficiency transcription vector pBS KS⁺ AMV-pA50
(Nowak et al., 1995). This plasmid was modified by
adding the recognition sequence for the restriction
enzyme SrfI downstream of the poly A sequence in the
plasmid. The new plasmid was designated M52. Subcloning
involved the isolation of a 1.1 kb NcoI/EcoRI restriction
fragment encoding the entire hGALR3 gene followed by its
ligation into NcoI/EcoRI digested M52. After
identification of a suitable clone (M54), the
transcription template was produced by linearization of
the plasmid DNA with SrfI. The plasmid M54 was deposited
on September 30, 1997, with the American Type Culture
Collection (ATCC), 12301 Parklawn Drive, Rockville,
Maryland 20852, U.S.A. under the provisions of the
Budapest Treaty for the International Recognition of the
Deposit of Microorganisms for the Purposes of Patent
Procedure and was accorded ATCC Accession No. 209312.
mRNAs were transcribed using the T7 polymerase ("Message
Machine", Ambion). Each oocyte received 2 ng each of
GIRK1 and GIRK4 mRNA in combination with 25 ng of GalR3
mRNA. In other experiments oocytes received injections
of mRNAs encoding the human α1A adrenergic receptor,

rGalR1 or rGalR2 galanin receptors (Forray et al., 1994; Parker et al., 1995) with or without GIRKs 1 and 4. After injection of mRNAs, oocytes were incubated at 17° for 3-8 days.

5

Dual electrode voltage clamp ("GeneClamp", Axon Instruments Inc., Foster City, CA) was performed as described above, with the following modifications: during recordings, oocytes were bathed in continuously flowing (1-3 mL/min) ND96 medium or, in the case of oocytes expressing GIRKs 1 and 4, elevated K^+ containing 48 mM KCl, 49 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (1/2hK). Drugs were applied either by local perfusion from a 10 μl glass capillary tube fixed at a distance of 0.5 mm from the oocyte, or for calculation of steady-state EC_{50}s, by switching from a series of gravity fed perfusion lines. Experiments were carried out at room temperature. All values are expressed as mean $\pm$ standard error of the mean.

20

MAP kinase
MAP kinase (mitogen activated kinase) may be monitored to evaluate receptor activation. MAP kinase is activated by multiple pathways in the cell. A primary mode of activation involves the ras/raf/MEK/MAP kinase pathway. Growth factor (tyrosine kinase) receptors feed into this pathway via SHC/Grb-2/SOS/ras. Gi coupled receptors are also known to activate ras and subsequently produce an activation of MAP kinase. Receptors that activate phospholipase C (Gq and G11) produce diacylglycerol (DAG) as a consequence of phosphatidyl inositol hydrolysis. DAG activates protein kinase C which in turn phosphorylates MAP kinase.

35 MAP kinase activation can be detected by several approaches. One approach is based on an evaluation of the phosphorylation state, either unphosphorylated

(inactive)   or   phosphorylated   (active).   The
phosphorylated protein has a slower mobility in SDS-PAGE
and can therefore be compared with the unstimulated
protein using Western blotting. Alternatively, antibodies
5   specific for the phosphorylated protein are available
(New England Biolabs) which can be used to detect an
increase in the phosphorylated kinase. In either method,
cells are stimulated with the mitogen and then extracted
with Laemmli buffer. The soluble fraction is applied to
10   an   SDS-PAGE   gel   and   proteins   are   transferred
electrophoretically   to   nitrocellulose   or   Immobilon.
Immunoreactive bands are detected by standard Western
blotting technique. Visible or chemiluminescent signals
are   recorded   on   film   and   may   be   quantified   by
15   densitometry.

Another approach is based on evaluation of the MAP kinase
activity via a phosphorylation assay. Briefly, cells are
stimulated with the mitogen and a soluble extract is
20   prepared. The extract is incubated at 30°C for 10 min
with gamma-32-ATP, an ATP regenerating system, and a
specific substrate for MAP kinase such as phosphorylated
heat and acid stable protein regulated by insulin, or
PHAS-I. The reaction is terminated by the addition of
25   H_3PO_4 and samples are transferred to ice. An aliquot is
spotted onto Whatman P81 chromatography paper, which
retains the phosphorylated protein. The chromatrography
paper is washed and counted for ^{32}P in a liquid
scintillation counter. Alternatively, the cell extract
30   is incubated with gamma-32-ATP, an ATP regenerating
system, and biotinylated myelin basic protein bound by
streptavidin to a filter support. The myelin basic
protein is a substrate for activated MAP kinase. The
phosphorylation reaction is carrried out for 10 min at
35   30°C. The extract can then by aspirated through the
filter, which retains the phosphorylated myelin basic
protein. The filter is washed and counted for ^{32}P by

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liquid scintillation counting.

Cell Proliferation Assay

Receptor activation of a G protein coupled receptor may
lead to a mitogenic or proliferative response which can
be monitored via ^{3}H-thymidine uptake. When cultured cells
are incubated with ^{3}H-thymidine, the thymidine
translocates into the nuclei where it is phosphorylated
to thymidine triphosphate. The nucleotide triphosphate
is then incorporated into the cellular DNA at a rate that
is proportional to the rate of cell growth. Typically,
cells are grown in culture for 1-3 days. Cells are forced
into quiescence by the removal of serum for 24 hrs. A
mitogenic agent is then added to the media. 24 hrs later,
the cells are incubated with ^{3}H-thymidine at specific
activities ranging from 1 to 10 uCi/ml for 2-6 hrs.
Harvesting procedures may involve trypsinization and
trapping of cells by filtration over GF/C filters with or
without a prior incubation in TCA to extract soluble
thymidine. The filters are processed with scintillant
and counted for ^{3}H by liquid scintillation counting.
Alternatively, adherant cells are fixed in MeOH or TCA,
washed in water, and solubilized in 0.05%
deoxycholate/0.1 N NaOH. The soluble extract is
transferred to scintillation vials and counted for ^{3}H by
liquid scintillation counting.

Tissue preparation for neuroanatomical studies

Male Sprague-Dawley rats (Charles River, Wilmington,
Massachusetts) are decapitated and the brains rapidly
removed and frozen in isopentane. Coronal sections may
be cut at 11 μm on a cryostat and thaw-mounted onto poly-
L-lysine coated slides and stored at -80°C until use.
Prior to hybridization, tissues are fixed in 4%
paraformaldehyde, treated with 5 mM dithiothreitol,
acetylated in 0.1 M triethanolamine containing 0.25%
acetic anhydride, delipidated with chloroform, and

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dehydrated in graded ethanols.

<u>Probes</u>

Oligonucleotide probes employed to characterize the
distribution of the rat GALR3 receptor mRNA may be
synthesized, for example, on a Millipore Expedite 8909
Nucleic Acid Synthesis System. The probes are then
lyophilized, reconstituted in sterile water, and purified
on a 12% polyacrylamide denaturing gel. The purified
probes are again reconstituted to a concentration of 100
ng/μL, and stored at -20°C. Probe sequences may include
DNA or RNA which is complementary to the mRNA which
encodes the GALR3 receptor.

<u>In Situ Hybridization</u>

Probes are 3'-end labeled with ^{35}S-dATP (1200 Ci/mmol, New
England Nuclear, Boston, MA) to a specific activity of
about 10^9 dpm/μg using terminal deoxynucleotidyl
transferase (Pharmacia). The radiolabeled probes are
purified on Biospin 6 chromatography columns (Bio-Rad;
Richmond, CA), and diluted in hybridization buffer to a
concentration of 1.5 x 10^4 cpm/μL. The hybridization
buffer consists of 50% formamide, 4X sodium citrate
buffer (1X SSC = 0.15 M NaCl and 0.015 M sodium citrate),
1X Denhardt's solution (0.2% polyvinylpyrrolidine, 0.2%
Ficoll, 0.2% bovine serum albumin), 50 mM dithiothreitol,
0.5 mg/ml salmon sperm DNA, 0.5 mg/ml yeast tRNA, and 10%
dextran sulfate. About one hundred μL of the diluted
radiolabeled probe is applied to each section, which is
then covered with a Parafilm coverslip. Hybridization is
carried out overnight in humid chambers at 40 to 55°C.
The following day the sections are washed in two changes
of 2X SSC for one hour at room temperature, in 2X SSC for
30 min at 50-60°C, and finally in 0.1X SSC for 30 min at
room temperature. Tissues are dehydrated in graded
ethanols and apposed to Kodak XAR-5 film for 3 days to 3
weeks at -20°C, then dipped in Kodak NTB3 autoradiography

emulsion diluted 1:1 with 0.2% glycerol water. After exposure at 4°C for 2 to 8 weeks, the slides are developed in Kodak D-19 developer, fixed, and counterstained with cresyl violet.

Solution hybridization/ribonuclease protection assay

For solution hybridization 2-15 μg of total RNA isolated from tissues may be used. Sense RNA synthesized using the full-length coding sequence of the rGalR2 is used to characterize specific hybridization. Negative controls may consist of 30 μg transfer RNA (tRNA) or no tissue blanks. Samples are placed in 1.5-ml microfuge tubes and vacuum dried. Hybridization buffer (40 μl of 400 mM NaCl, 20 mM Tris, pH 6.4, 2 mM EDTA, in 80% formamide) containing 0.25-1.0 X 10^6 counts of each probe is added to each tube. Samples are heated at 90°C for 15 min, after which the temperature is lowered to 45°C for hybridization.

After hybridization for 14-18 hr, the RNA/probe mixtures are digested with RNAse A (Sigma) and RNAse T1 (Bethesda Research Labs, Gaithersburg, Maryland). A mixture of 2.0 μg RNAse A and 1000 units of RNAse T1 in a buffer containing 330 mM NaCl, 10 mM Tris (pH 8.0) and 5 mM EDTA (400 μl) is added to each sample and incubated for 90 min at room temperature. After digestion with RNAses, 20 μl of 10% SDS and 50 μg proteinase K are added to each tube and incubated at 37°C for 15 min. Samples are then extracted with phenol/chloroform:isoamyl alcohol and precipitated in 2 volumes of ethanol for 1 hr at -70°C. tRNA is added to each tube (30 mg) as a carrier to facilitate precipitation. Following precipitation, samples are centrifuged, washed with cold 70% ethanol, and vacuum dried. Samples are dissolved in formamide loading buffer and size-fractionated on a urea/acrylamide sequencing gel (7.6 M urea, 6% acrylamide in Tris-borate-EDTA). Gels are dried and apposed to Kodak XAR-5 x-ray

film.

Development of probes: Using full length cDNA encoding
the rat Gal R3 receptor as a template, PCR was used to
5   amplify a 455 base pair fragment corresponding to
nucleotides 1061-1516 of the coding sequence. Primers
used in PCR contained both sp6 and T7 RNA polymerase
promoter sequences, and the PCR generated fragments were
subcloned into a plasmid vector (pUC-18). This construct
10   was linearized with Bam HI or Hind III. sp6 and T7 RNA
polymerases were used to synthesize the sense and
antisense strands of RNA respectively. Full length RNA
transcripts were obtained using a full length cDNA
construct in pBluescript.

15

A probe coding for rat glyceraldehyde 3-phosphate
dehydrogenase (GAPDH) gene, a constitutively expressed
protein, is used concurrently. GAPDH is expressed at a
relatively constant level in most tissue and its
20   detection is used to compare expression levels of the rat
GalR3 gene in different tissue.

Extraction of RNA: Tissue harvested from rat peripheral
tissue as well as regions of the CNS was frozen using
25   liquid N_2 and stored at -70°C until needed Tissue was
homogenized in buffer containing detergent, protein and
RNase degrader. The homogenate was incubated with
Oligo(dT) cellulose powder, and washed extensively. mRNA
was eluted from the Oligo(dT) cellulose with 10 mM Tris,
30   and precipitated after the addition of NaCl. Yield and
relative purity were assessed by measuring absorbance
A_{260}/A_{280}.

Synthesis of probes: rGALR3 and GAPDH cDNA sequences
35   preceded by phage polymerase promoter sequences were used
to synthesize radiolabeled riboprobes. Conditions for
the synthesis of riboprobes were: 0.5-1.0 μL linearized

template (1µg/µL), 1.5 µL of ATP, GTP, UTP (10 mM each),
3 µL dithiothreitol (0.1 M), 30 units RNAsin RNAse
inhibitor, 0.5-1.0 µL (15-20 units/µL) RNA polymerase,
7.0 µL transcription buffer (Promega Corp.), and 12.5 µL
5   α^{32}P-CTP (specific activity 3,000Ci/mmol). 0.1 mM CTP
(0.02-1.0 µL) were added to the reactions, and the volume
were adjusted to 35 µL with DEPC-treated water. Labeling
reactions were incubated at 37°C for 90 min, after which
3 units of RQ1 RNAse-free DNAse (Promega Corp.) were
10   added to digest the template. The riboprobes were
separated from unincorporated nucleotide by a spun G-50
column (Select D G-50(RF); 5 Prime-3 Prime, Inc.). TCA
precipitation and liquid scintillation spectrometry were
used to measure the amount of label incorporated into the
15   probe. A fraction of all riboprobes synthesized were
size-fractionated on 0.4 mm thick 5% acrylamide
sequencing gels and autoradiographed to confirm that the
probes synthesized were full-length and not degraded.

20   **Solution hybridization/ribonuclease protection assay:**
For solution hybridization 2.0 µg of total RNA isolated
from tissues were used. Sense RNA synthesized using the
full-length coding sequence of the rGalR3 was used to
characterize specific hybridization. Negative controls
25   consisted of 30 µg transfer RNA (tRNA) or no tissue
blanks. All mRNA samples were placed in 1.5-ml microfuge
tubes and vacuum dried. Hybridization buffer (40 µl of
400 mM NaCl, 20 mM Tris, pH 6.4, 2 mM EDTA, in 80%
formamide) containing $0.25\text{-}1.0 \times 10^6$ counts of each probe
30   were added to each tube. Samples were heated at 90°C for
15 min, after which the temperature was lowered to 42°C
for hybridization.

After hybridization for 14-18 hr, the RNA/probe mixtures
35   were digested with RNAse A (Sigma) and RNAse T1 (Life
Technologies). A mixture of 2.0 µg RNAse A and 1000
units of RNAse T1 in a buffer containing 330 mM NaCl, 10

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mM Tris (pH 8.0) and 5 mM EDTA (400 μL) was added to each
sample and incubated for 90 min at room temperature.
After digestion with RNAses, 20 μL of 10% SDS and 50 μg
proteinase K were added to each tube and incubated at
5   37°C for 15 min.   Samples were then extracted with
phenol/chloroform:isoamyl alcohol and precipitated in 2
volumes of ethanol for 1 hr at -70°C.   Pellet Paint
(Novagen) was added to each tube (2.0 μg) as a carrier to
facilitate precipitation.   Following precipitation,
10   samples were centrifuged, washed with cold 70% ethanol,
and vacuum dried.   Samples were dissolved in formamide
loading buffer and size-fractionated on a urea/acrylamide
sequencing gel (7.6 M urea, 6% acrylamide in Tris-borate-
EDTA).   Gels were dried and apposed to Kodak XAR-5 x-ray
15   or BioMax film and exposed at -70°C.

In vivo methods
The effects of galanin, galanin derivatives, and
related peptides and compounds may be evaluated by
20   intracerebroventricular (i.c.v.) injection of the
peptide or compound followed by measurement of food
intake in the animal. Measurement of food intake was
performed for 3 hours after injection, but other
protocols may also be used. Saline was injected as a
25   control, but it is understood that other vehicles may
be required as controls for some peptides and
compounds. In order to determine whether a compound is
a GALR3 antagonist, food intake in rats may be
stimulated by administration of (for example) a galanin
30   receptor agonist through an intracerebroventricular
(i.c.v.) cannula. A preferred anatomic location for
injection is the hypothalamus, in particular, the
paraventricular nucleus. Methods of cannulation and
food intake measurements are well-known in the art, as
35   are i.c.v. modes of administration (Kyrkouli et al.,
1990, Ogren et al., 1992). To determine whether a
compound reduces agonist-stimulated food intake, the

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compound may be administered either simultaneously with the peptide, or separately, either through cannula, or by subcutaneous, intramuscular, or intraperitoneal injection, or more preferably, orally.

Materials

Cell culture media and supplements are from Specialty Media (Lavallette, NJ). Cell culture plates (150 mm and 96-well microtiter) are from Corning (Corning, NY). Sf9, Sf21, and High Five insect cells, as well as the baculovirus transfer plasmid, pBlueBacIII™, are purchased from Invitrogen (San Diego, CA). TMN-FH insect medium complemented with 10% fetal calf serum, and the baculovirus DNA, BaculoGold™, is obtained from Pharmingen (San Diego, CA.). Ex-Cell 400™ medium with L-Glutamine is purchased from JRH Scientific. Polypropylene 96-well microtiter plates are from Co-star (Cambridge, MA). All radioligands are from New England Nuclear (Boston, MA).

Galanin and related peptide analogs were either from Bachem California (Torrance, CA), Peninsula (Belmont, CA); or were synthesized by custom order from Chiron Mimotopes Peptide Systems (San Diego, CA).

Bio-Rad Reagent was from Bio-Rad (Hercules, CA). Bovine serum albumin (ultra-fat free, A-7511) was from Sigma (St. Louis. MO). All other materials were reagent grade.

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Experimental Results

Isolation of a partial GALR3 cDNA from rat hypothalamus

In order to clone additional members of the galanin
5   receptor family, a homology cloning strategy based on
the potential presence of multiple galanin receptors in
hypothalamus was designed. Although recent evidence
indicated that GALR1 and GALR2 receptor mRNAs were
present in rat hypothalamus (Gustafson et al., 1996;
10   Parker et al., 1995), not all aspects of the cloned
GALR1 and GALR2 pharmacological profiles match that
observed for galanin-mediated feeding (Crawley et al.,
.1993). These results suggested that the regulation of
galanin-induced feeding may not be explained by the
15   presence of only GALR1 or GALR2 (or both) in the rat
hypothalamus.

In order to attempt to isolate additional galanin
receptors, a rat hypothalamus cDNA phage library was
20   screened, under reduced stringency conditions, with
oligonucleotide probes directed to the transmembrane
regions of the rat GALR2 neuropeptide receptor gene.
Five positively-hybridizing clones were isolated,
plaque-purified and characterized by Southern blot
25   analysis and sequencing. One clone, rHY35a, contained
a 3.5 kb insert (consisting of a 1.0kb, 0.2kb, and
2.3kb EcoRI fragments), which hybridized with the
second transmembrane domain oligonucleotide probe of
rat GALR2. DNA sequence analysis indicated greatest
30   homology to the published rat GALR1 gene (Burgevin, et
al., 1995) and the novel rat GALR2 receptor gene we
have recently identified. This clone was a partial
intronless gene fragment, containing an open reading
frame and encoding a predicted starting MET through the
35   middle of the predicted seventh transmembrane domain,
with ≈150 nucleotides of 5' UT. Hydropathy analysis
of the predicted translated protein is consistent with

a putative topography of at least six transmembrane
domains (the predicted sequence ended in the middle of
TM7), indicative of the G protein-coupled receptor
family. This gene fragment exhibited 52% and 66%
5   nucleotide identity and 37% and 60% amino acid identity
to the rat GALR1 and rat GALR2 receptors, respectively.
Furthermore, PCR primers directed to the amino terminus
(forward primer) and first extracellular loop (reverse
primer) of each of the corresponding receptor genes,
10   rGALR1 and rGALR2, were unable to amplify this clone,
whereas primers directed to this clone resulted in the
correct size PCR product. The putative six (or seven)
transmembrane topography and the high degree of
identity to rat GALR1 and GALR2 suggested that this
15   cDNA represented a partial gene fragment of a novel
galanin-like receptor gene, referred to herein as
GALR3.

In order to obtain the full-length gene, PCR on cDNA
20   derived from the RIN14B cell line, using internal
primers directed to TM3 and third intracellular loop of
rat GALR3 was first conducted. It was hypothesized
that since previous data indicated that this cell line
expressed both GALR1 and GALR2, it may also contain
25   further subtypes. PCR analyses revealed the presence
of at least a portion of GALR3 in cDNA from RIN14B
cells; the absence of reverse transcriptase did not
result in PCR amplification, indicating the ability to
amplify RIN14B cDNA was due to authentic GALR3 mRNA and
30   not any contaminating genomic DNA in the RNA source.

To isolate a cDNA molecule from RIN14B which expresses
GALR3, a RIN14B plasmid library was screened by PCR
(using internal primers) and two pools, F105 and F212,
35   were identified which contained a PCR product of the
correct size. To determine if the insert was in the
correct orientation for expression and to determine the

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size of the cDNA insert (including the coding region,
5'UT and 3'UT), vector-anchored PCR was conducted on
each pool. The PCR analyses suggested that both pools
contained full-length GALR3 but in the incorrect
5   orientation and thus would be predicted not to express
the GALR3 receptor. Examination of slides of COS-7
cells which had been transfected with DNA from each of
these pools and subsequently bound with radioligand
confirmed the absence of binding of radiolabeled
10 galanin, presumably due to its incorrect orientation.

Although the full-length clone of rat GALR3 in the
correct orientation from the RIN14B plasmid library was
not obtained, it was reasoned that the sequence of the
15 missing 3' end (i.e., from the middle of TM7 through
the stop codon) could be obtained by sequencing the
vector-anchored PCR product corresponding to the 3' end
of the molecule. An ≈1.2 kb PCR product from a vector-
anchored amplification of bacterial glycerol stock of
20 the F105 pool was obtained, using a vector-derived
reverse primer and a rGALR3-specific forward primer
from TM6. This PCR product was sequenced with the
gene-specific primer to reveal an overlap within TM7
with the sequence known from rHY35a. In addition,
25 further sequence was obtained representing an open
reading frame corresponding to the missing second half
of TM7 and the carboxy terminus. The sequence obtained
showed an overall 47% nucleotide identity to rGalR2,
and a 62% nucleotide identity to rGalR2 from the third
30 extracellular domain to the 5' end of the COOH
terminus, confirming the existence of an open reading
frame from a starting MET through a stop codon, with
the presence of seven putative transmembrane domains.
Furthermore, this sequence permitted us to design an
35 oligonucleotide primer in the 3' UT which could serve
as a diagnostic tool for determination of full-length
characterization of additional pools of DNA (see

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below).

Since the most convenient method to obtain the full-
length rGALR3 clone in the correct orientation in an
5   expression vector is to locate a full-length clone in
preexisting libraries, and it was known that this gene
was expressed in rat hypothalamus, we screened a rat
hypothalamus plasmid library ("K") by PCR. Two
superpools from the K library (#3 and #17) were
10   identified as containing rGALR3. A primary pool, K163
(from superpool #17), was identified to be positive and
full-length using internal and full-length PCR primers,
and vector-anchor primers were used to determine the
orientation. These data were consistent with primary
15   pool K163 (made up of 3200 primary clones), containing
full-length rGALR3 in the correct orientation in the
expression vector, pEXJ.T7. Furthermore, this pool
failed to amplify with GALR1- and GALR2- specific
primers and yet exhibited galanin binding when DNA from
20   this pool was used to transfect COS cells and tested
for radiolabeled galanin binding. These data suggested
that a pool from a rat hypothalamus plasmid cDNA
library which contains the novel sequence initially
identified from rat hypothalamus as a galanin-like
25   receptor had been identified, which, in addition,
exhibits galanin binding, thereby identifying the pool
as containing a novel galanin receptor, referred to
herein as GALR3, or more specifically, rGALR3.

30   The pool K163 was then sib selected through one round
by PCR and a second round by colony hybridization,
using a probe directed to the amino terminus of the
sequence from rHY35a, resulting in the isolation of a
single clone (i.e., a bacterial colony containing rat
35   GALR3), called K163-30-17, representing the full-length
rat GALR3 in the correct orientation. The rGALR3
recombinant bacterial colony was grown up in broth with

ampicillin and DNA extracted. Restriction enzyme
digestion suggested a 2.1 kb insert, consistent with
the clone comprising the full-length coding region.

5   Furthermore, sequence analysis on K163-30-17 DNA
(plasmid K1086) confirmed that it contained a full-
length coding region in the correct orientation for
expression.

10   <u>Northern Blot Analyses of GALR3 mRNA</u>
To define the size and distribution of the mRNA
encoding GALR3, Northern blot analyses of poly A⁺ RNA
from various rat tissues and brain regions was carried
out. A radiolabeled 70-mer oligonucleotide probe
15   directed to the amino terminus of the rat GALR3 coding
region was used as a hybridization probe under high
stringency. This probe failed to cross-hybridize with
either the GALR1 or GALR2 genes under similar
hybrization conditions, demonstrating its specificity
20   for GALR3 receptor. A single transcript of ≈3.3 kb is
detected after a 5 day exposure of the autoradiogram at
-80°C. using Kodak Biomax MS film with a Biomax MS
intensifying screen. GALR3 mRNA was not detected by
Northern analysis in the brain nor in various regions
25   of the brain (see Table 1). Among various rat tissues,
the GALR3 transcript had a restricted distribution;
GALR3 mRNA was predominantly observed in kidney with a
faint signal detected in liver (see Table 1). This
distribution was the same upon a longer exposure of the
30   autoradiogram (14 days). Northern blots were reprobed
with G3PDH probe to assess whether similar amounts of
mRNA were present in each lane.

Northern blot analyses of poly A+ RNA from various
35   human brain regions and peripheral tissues were carried
out with a radiolabeled 70-mer oligonucleotide probe
directed to the amino terminus of the human GALR3

coding region under high stringency. As demonstrated
for the corresponding rat probe, this human probe
failed to cross-hybridize with either the human GALR1
or GALR2 genes under similar hybridization conditions,

5   demonstrating its specificity for human GALR3 receptor.
No transcript was observed even after 14 day exposure
of the autoradiogram in any of the human brain regions
or peripheral tissues, by Northern blot analyses. The
regions of the brain and periphery included in this

10   analysis, as contained in the MTN blots from Clontech,
included: amygdala, caudate nucleus, corpus callosum,
hippocampus, total brain, substantia nigra, subthalamic
.brain, thalamus nucleus, cerebellum,cerebral cortex,
medulla, spinal cord, occipital pole, frontal lobe,

15   temporal lobe, putamen, heart, total brain, placenta,
lung, liver, skeletal muscle, kidney, and pancreas.

Reverse-transcription PCR of GALR3 mRNA
Amplification of cDNA derived from mRNA of various rat

20   peripheral and brain regions demonstrated the presence
of GALR3 mRNA in various regions of the brain,
including hypothalamus (see Table 2), as well as
several peripheral tissues tested, such as pancreas and
liver. It was anticipated that we would identify GALR3

25   mRNA in hypothalamus since the gene was cloned from
this region of the brain (supra). Therapeutic
indications implied from localization of GALR3 mRNA for
several of these regions are also indicated in Table
2.

Table 1. Northern blot analyses of GALR3 mRNA in brain
and various peripheral rat tissues.

Tissue	Intensity of Signal	Therapeutic Indications
Heart	(-)	
Brain	(-)	
Spleen	(-)	
Lung	(-)	
Liver	+	Diabetes
Skeletal Muscle	(-)	
Kidney	++	Hypertension, electrolyte balance, diuretic, anti-diuretic
Testis	(-)	
Spinal cord	(-)	
Periaqueductal Grey	(-)	
Cerebellum	(-)	
Cortex	(-)	
Brain Stem	(-)	
Hypothalamus	(-)	
Amygdala	(-)	
RIN14B cell line	(-)	

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Table 2.   RT-PCR analyses of GALR3 mRNA in brain and various peripheral rat tissues.

Tissue	Intensity of Signal	Therapeutic Indications
Heart	(-)	
Brain	+	Obesity/feeding, analgesia, cognition enhancement, Alzheimer's disease, depression, anxiety, sleep disorders, Parkinson's disease, traumatic brian injury, convulsion/epilepsy
Spleen	+	Immune functions, hematopoiesis
Lung	+	Respiratory disorders, asthma, emphysema, lung cancer diagnostics
Liver	+	Diabetes
Skeletal Muscle	(-)	Diabetes
Smooth Muscle	+	
Kidney	+	Hypertension, electrolyte balance, diuretic, anti-diuretic
Pancreas	+++	Appetite/obesity, diabetes, gastrointestinal disorders, neuroendocrine regulation
Retina	(-)	
Testis	+	Reproductive function
Ventral spinal cord	++	Analgesia
Dorsal spinal cord	++	Analgesia
Periaqueductal Grey	(-)	

Cerebellum	+	Motor disorders
Cortex	(-)	
Brain Stem	+	Autonomic disorders
Lower midbrain	+	
Hypothalamus	++	Neuroendocrine regulation, appetite/obesity
Amygdala	(-)	
RIN14B cell line	+	Neuroendocrine regulation, including diabetes

RNase protection assay to detect mRNA coding for rat GALR3

mRNA was isolated and assayed as described from: heart, striated muscle, liver, kidney, lung, stomach, spleen, pancreas, pituitary, adrenal medulla, adrenal cortex, trigeminal ganglion and CNS regions. CNS regions included: whole brain, spinal cord, medulla, hypothalamus, cerebral cortex, cerebellum, hippocampus, caudate-putamen, and substantia nigra. Levels of rat GALR3 mRNA were extremely low in all areas assayed. The highest levels of rat GALR3 mRNA were detected in the hypothalamus. Lower amounts were found in: kidney, liver, stomach, pancreas, spleen, pituitary, adrenal medulla, adrenal cortex, whole brain, spinal cord, medulla, cerebellum and caudate/putamen. At the present time, mRNA coding for the rat GALR3 has not been detected in RNA extracted from other regions (Table 3).

Table 3. Distribution of mRNA coding for rat GALR3 receptors.

Region	rGalR3	Potential applications
liver	+	Diabetes

kidney	+	Hypertension, Electrolyte balance
lung	+	Respiratory disorders, asthma
heart	−	Cardiovascular indications
stomach	+	Gastrointestinal disorders
duodenum	−	Gastrointestinal disorders
spleen	+	Immune function
pancreas	+	Diabetes, endocrine disorders
testicle	−	Reproductive function
striated muscle	−	Musculoskeletal disorders, diabetes
pituitary	+	Endocrine/neuroendocrine regulation
adrenal medulla	+	Regulation of epinephrine release
adrenal cortex	+	Regulation of steroid hormones
trigeminal ganglion	−	Analgesia, sensory transmission, migraine
whole brain	+	
cerebral cortex	+	Sensory integration, cognition
hypothalamus	++	Appetite/obesity, Neuroendocrine regulation
hippocampus	−	Cognition/memory
spinal cord	++	Analgesia, sensory modulation and transmission
cerebellum	+	Motor coordination
medulla	+	Analgesia, motor coordination
substantia nigra	−	Modulation of dopaminergic function. Modulation of motor coordiantion.
caudate-putamen	+	Modulation of dopaminergic function

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Pharmacological characterization of GALR3

The pharmacology of GALR3 was studied in COS-7 cells
transiently transfected with the GALR3 cDNA, K163-30-17
(or "K1086"). COS-7 cells transfected with the single
5   clone K1086 exhibit specific binding of ^{125}I-galanin in
comparison with COS-7 cells transfected with control
vector. In preliminary radioligand binding
experiments, porcine ^{125}I-galanin bound to membranes
from COS-7 cells transfected with K1086, with a
10   specific binding of 90 fmol/mg, when the membranes
(0.17 mg/mL) were incubated with 2.1 nM porcine ^{125}I-
galanin for 60 min at room temperature. (Specific
binding was decreased by as much as 70% when the
incubation temperature was raised to 30°C, suggesting
15   receptor instability and/or protease activity in the
membrane preparation.) In this experiment, the binding
buffer used was that described for the whole cell slide
binding assay. No specific binding was detected to
membranes from mock-transfected COS-7 cells when tested
20   under the same conditions.

In another experiment, COS-7 cells were transiently
transfected with a "trimmed" plasmid (designated pEXJ-
RGalR3T), which comprises the entire coding region of
25   rat GALR3, but in which the 5' initiating ATG is joined
directly to the vector, and which comprises only 100
nucleotides from the 3' untranslated region, after the
stop codon (i.e., up to and including nucleotide 1275
in Figure 1). A full saturation binding analysis using
30   ^{125}I-galanin was performed using the COS-7 cells
transfected with plasmid pEXJ-RGalR3T, and yielded a K_d
(dissociation constant) of 0.34 nM and an apparent B_{max}
as high as 570 fmol/mg. The use of the "trimmed"
plasmid provides for greater expression and therefore
35   greater convenience and accuracy in binding assays.

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   Peptide displacement assays yielded a distinct rank
   order of binding affinity (Table 4). Porcine galanin
   bound with relatively high affinity (K_i = 5 nM), C-
   terminal truncation to porcine galanin 1-16 was
5 disruptive (Ki = 86 nM), and galanin 3-29 as well as D-
   Trp^2-galanin analogs were without demonstrable binding.
   Two chimeric peptides displayed high affinity for
   GALR3 (M32 and M35) whereas galantide was slightly less
   active and the putative "antagonists" C7 and M40 were
10 relatively weak ligands.

   Peptide binding profiles for the rat GALR1, GALR2 and
   GALR3 receptor subtypes were derived from membranes
   prepared from transiently transfected COS-7 cells.
15 Rat GALR3 is distinguished from the other receptor
   subtypes by having 40-fold lower affinity for M40 vs.
   galanin, whereas the rat GALR1 and GALR2 receptor
   subtypes display <= 8-fold lower affinity for M40 vs.
   galanin. Rat GALR3 also displays low affinity for the
20 D-Trp^2-galanin analogs, which appear to be primarily
   useful for distinguishing the rat GALR2 receptor. It
   is concluded that the rat GALR3 displays a distinctive
   pharmacological profile which can be used to evaluate
   receptor expression in native cells and tissues.

25

Table 4. Peptide binding profile of rat GALR1, GALR2
and GALR3 receptors transiently expressed in COS-7 cell
membranes and labeled with porcine ^{125}I-galanin. Values
are reported as K_i (nM).

Peptide	GALR1 (K_i, nM)	GALR2 (K_i, nM)	GALR3 (K_i, nM)
porcine galanin	0.46	0.45	5.1
M32	0.62	12	2.1
M35	0.33	0.57	6.7
galantide	9.5	2.0	18
C7	16	19	68
M40	3.6	0.72	210
porcine galanin 1-16	2.2	7.2	86
D-Trp[2]-galanin 1-29	3700	52	> 1000
D-Trp[2]-galanin 1-16	40 000	23	> 1000
porcine galanin 3-29	> 100 000	> 100 000	> 1000

Isolation of the human GALR3 gene

A human placenta genomic library in λ dash II (≈1.5 x
10[6] total recombinants) was screened using the same set
of overlapping oligonucleotide probes to TM regions 1-7
of rat GALR2 and under the same hybridization and wash
conditions as described for screening the rat
hypothalamus cDNA library. Lambda phage clones
hybridizing with the probe were plaque purified and DNA
was prepared for Southern blot analysis. One phage
clone, plc21a, contained a 2.7 kb KpnI/EcoRI fragment
which hybridized with the rat GALR2 TM2 oligonucleotide

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probe and was subsequently subcloned into a pUC vector
for sequence analysis. The cloned human genomic
fragment contains an a open reading frame from the
starting MET codon to a predicted intron in the second
5 intracellular loop, with a nucleotide identity of 88%
(93% aa identity) with the rat GALR3 receptor described
above (thus establishing this human genomic clone to be
the human homologue of rat GALR3). Although this human
genomic fragment was not full-length and contained an
10 intron downstream of TM3, it is anticipated that the
full-length, intronless version of the human GALR3
receptor gene may be isolated using standard molecular
biology techniques, as described in Materials and
Methods.

15

Since the human genomic fragment was not full-length
and contained an intron downstream of TM3, it was
hypothesized that the original phage clone, which
contains an average insert size of about 18kb, may
20 contain the 3' end of this gene, assuming a smaller
size for the intron which serparates the 5' and 3'
exons. The presence of the exon, representing the 3'
end of the human GALR3, on the original phage clone,
was demonstrated by positive hybridization signals of
25 the phage clone, plc21a, with probes directed to the
third extracellular loop or TM4 of the rat GALR3 gene.

The full-length human GALR3 gene was constructed by
ligating a PCR-derived product of the 5' exon,
representing the starting MET through the 3/4 loop with
30 a synthetically-created KpnI site appended to the
reverse PCR primer, and the 3' exon, contained on a 1.4
kb KpnI genomic fragment. The full-length human GALR3
gene contains 1107 bp within its coding region,
encoding for a predicted protein of 368 aa. The rat
35 homologue contains two additional aa and encodes for a

predicted protein of 370 aa. The human and rat GALR3
homologues exhibit 86% nucleotide and 92% amino acid
identities, consistent with designating these genes as
species homologues of the same gene within the GPCR

5   family. The amino acid identity increases to 96% when
restricting the comparison to within the transmembrane
domains. The human GALR3 gene exhibits 52% and 67%
nucleotide identities and 36% and 58% amino acid
identities to the human GALR1 and GALR2 receptors,

10 respectively. Furthermore, within the transmembrane
domains, the human GALR3 receptor displays 46% and 74%
amino acid identities with the human GALR1 and GALR2
receptors, respectively. This relationship suggests
that human GALR3 represents a novel receptor subtype

15 within the galanin gene family.

Pharmacological characterization of human GALR3

The pharmacology of human GALR3 was studied in COS-7
cells transiently transfected with pEXJ-hGalR3. In

20 preliminary radioligand binding experiments using
membranes prepared from COS-7 cells transfected with
pEXJ-hGalR3, specific binding of galanin was observed
with binding of 6 fmol/mg when the membranes (0.31
mg/mL) were incubated with 0.32 nM porcine ^{125}I-galanin

25 for 2 hrs. at room temperature. No mock transfection
was performed in this assay because no galanin binding
to COS-7 cells was observed previously in binding
experiments using similar conditions (supra).

30 In a subsequent experiment, when membranes from
transiently transfected cells (membrane protein = 0.15
mg/ml) were incubated with porcine ^{125}I-galanin (0.32
nM), specific binding was measured as 110 fmol/mg.
Therefore, it is concluded that the human GALR3

35 receptor cDNA leads to expression of functional GALR3
receptors, thereby providing an important tool with

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which to evaluate ligand selectivity for human GALR1, GALR2 and GALR3 receptor subtypes.

In further experiments, cell lines stably expressing
5   the rat and human GALR3 receptors were prepared. Membranes from the stably transfected cell line 293-rGalR3-105 bound porcine ^{125}I-galanin with a K_d of 0.74 nM and an apparent B_{max} of 450 fmol/mg membrane protein. Both the transiently and stably expressed rat GALR3
10   receptors were analyzed in competitive displacement assays using porcine ^{125}I-galanin (Table 5). Like GALR2, GALR3 appears to bind the N-terminally extended peptide galanin -7 to + 29 with affinity comparable to that for porcine galanin. These data provide a
15   pharmacological fingerprint which should be useful for characterizing GALR3-dependent processes in vivo.

Next, the cDNA for the human GALR3 receptor was used to prepare both transiently and stably transfected cells.
20   Membranes from COS-7 cells transiently transfected with human GALR3 cDNA bound porcine ^{125}I-galanin with a K_d of 1.25 nM and an apparent B_{max} of 750 fmol/mg membrane protein. Membranes LM(tk-) cells stably transfected with human GALR3 receptor cDNA (L-hGalR3-228) bound
25   porcine ^{125}I-galanin with a K_d of 2.57 nM and an apparent B_{max} of 1700 fmol/mg membrane protein. Specific binding measured in the presence of 0.3 nM porcine ^{125}I-galanin was reduced by 40% in the presence of nonhydrolyzable guanine nucleotides such as GTPγS or
30   Gpp(NH)p at concentrations up to 100 μM. These data suggest that the human GALR3 receptor interacts with one or more G proteins in the LMTK- cell, and furthermore, that receptor stimulation by galanin might lead to a functional response in the LMTK- cell at the
35   level of the G-protein or further downstream in the signal transduction pathway. Preliminary analyses in

peptide displacement assays using porcine ^{125}I-galanin
as the radioligand indicate that the human GALR3
receptor, sharing 92% amino acid identity with the rat
GALR3 receptor, binds galanin and related analogs with
affinities resembling those for the rat receptor. A
similar pharmacological profile for both the human and
rat GALR3 receptor homologs suggests that the rat may
be used to model the therapeutic value of GALR3-
directed ligands. A noteworthy feature of the
pharmacology is that the GALR3 receptor, whether human
or rat, binds human galanin with lower affinity
compared to rat and porcine galanin. Human galanin is
also somewhat less potent than porcine galanin in both
in vitro functional and in vivo feeding assays. This
relationship differentiates the GALR3 receptor from the
GALR1 and GALR2 subtypes, and may be useful in further
investigations.

Table 5.

Peptide	Ki (nM)			
	Rat GalR3 COS7	293-rGalR3-105	Human GalR3 COS7	L-hGalR3-228
M32	1.9	1.0		6.0
M35	3.7	3.2	19	7.8
rat galanin	4.3	5.7		
porcine galanin	5.1	5.8	5.3	14
human galanin	10.5	53	19	69
galantide	9.0	7.6	23	40
C-7	23	9.6		8.1
M40	103	85		130

porcine galanin 1-16	52	138	35 300	320
D-Trp2-galanin	> 1000	> 1000		
galanin -7 to + 29	3.3	21		29

Signal transduction pathway of hGalR3:   stimulation of K⁺ currents

Heterologous expression of GPCRs in *Xenopus* oocytes has been widely used to determine the identity of signaling pathways activated by agonist stimulation (Gundersen et al., 1983; Takahashi et al., 1987; Dascal et al., 1993). A large family of GPCRs that naturally couple to heterotrimeric G-proteins of the G_i/G_o class activate GIRK channels (North, 1989) in native neurons (Kofuji et al., 1995) and in the *Xenopus* expression system (Dascal et al., 1993; Kubo et al., 1993; Krapivinsky et al., 1995). Under voltage clamp conditions, oocytes injected with mRNAs for hGALR3 and GIRKs 1 and 4 responded with inward currents to local perfusion of porcine galanin (Fig. 6A). Average currents were 51.3 ± 9.4 nA (n = 16) in the presence of 1 μM porcine galanin, whereas oocytes injected with mRNAs for GIRKs 1 and 4 alone produced little or no inward current (2.5 ± 1.2 nA, n = 8) in response to 1 μM galanin. Oocytes injected with mRNA encoding the rat GalR3 receptor also exhibited current responses to the 1 μM local application of M32 or porcine galanin. The pharmacology of the rat GalR3 receptor was not further evaluated in oocytes. In oocytes expressing human GalR3, evidence that galanin-induced currents were mediated by GIRK channels included: 1) dependency on elevated external K⁺, 2) strong inward rectification of the current-voltage (I/V) relation, 3) reversal potential

(-26 ± -2 mV) close to the predicted equilibrium
potential for K^+ (-23 mV), 4) sensitivity to block by 300
μM Ba^{++} (Fig. 6A), and 5) lack of galanin-sensitivity in
oocytes injected with only hGALR3 mRNA (data not shown).

5   Currents having these same properties, but larger in
amplitude, were also evoked by galanin in oocytes
expressing GALR1 receptors in combination with GIRKs 1
and 4 (Table 6). Thus, GALR1 and GALR3 receptors appear
to have a related signal transduction pathway.

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Other GPCRs, when expressed in *Xenopus* oocytes, activate
a Ca^{++}-dependent Cl^- conductance that results from the
activation of phospholipase C and the subsequent release
of Ca^{++} from intracellular stores. This pathway was not

15   activated in oocytes expressing hGALR3 since Cl^- currents
were never observed following application of galanin (n
= 20). (Cl^- currents were also not observed in oocytes
expressing the GALR1 receptor.) In contrast, in oocytes
expressing mRNAs encoding GALR2 or α_{1a} receptors, 1 μM

20   galanin or epinephrine, respectively, stimulates
transient Cl^- currents (data not shown). To provide
further evidence that hGALR3 couples to the Go/Gi family
of G-proteins, batches of oocytes, previously injected
with hGALR3 and GIRK mRNAs, were injected with pertussis

25   toxin (2 ng/oocyte) and tested for receptor coupling to
K^+ currents. In oocytes treated with the toxin, galanin
currents were completely abolished (Fig. 7); oocytes
injected with buffer alone displayed normal galanin-
induced currents. A similar sensitivity to pertussis

30   toxin was observed for oocytes expressing GALR1
receptors. Agonist responses in oocytes expressing GALR2
or α_{1a} adrenergic receptors were unaffected by pertussis
toxin (Fig. 7, Table 6). Taken together, these results
support the conclusion that GALR1 and GALR3 receptors

35   couple to a Gi/Go pathway, and that GALR2 (like the α_{1a}
adrenergic receptor) couples to a G_q-type pathway (Table
7).

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Table 6.   Effects of pertussis toxin treatment on currents generated by stimulation of galanin or alpha adrenergic receptors expressed in oocytes. Current is presented in nA (nanoamperes).

	Receptor			
	rat GALR1	rat GALR2	human GALR3	Alpha 1a
Control	1775 ± 278	229 ± 60	24 ± 5	5483 ± 1154
PTX	17 ± 3	238 ± 51	0 ± 0	6350 ± 1318

Table 7.   Comparison of intracellular signaling pathways for three galanin receptors expressed in oocytes.

Receptor	Signaling pathway		
	Activates Cl⁻ current	Activates GIRKs	PTX sensitive
rGALR1	no	yes	yes
rGALR2	yes	no	no
hGALR3	no	yes	yes

Pharmacology of hGALR3 in oocytes

A series of galanin and galanin-related peptides were tested at the human GALR3 receptor for agonist and antagonist activities.   Of these peptides, porcine galanin, human galanin, M32, C7, M35, M15 (spantide), galanin -7-29, galanin 1-16, and M40 evoked agonist activity at a fixed dose of 1 μM.   D-Trp2-galanin and galanin 3-29 were inactive. EC_{50}s were constructed from cumulative concentration-response measurements performed on a series of oocytes (Figs. 6B, 8). EC_{50}s (in rank order) for M32, porcine galanin, C7, human galanin, galanin -7 to 29, galanin 1-16, and M40 were 45, 222, 343, 381, 1906, 2030, and 2265 nM, respectively (Table 8).   This rank order of potency was similar to that observed for K_i values in binding assays using the human GalR3 receptor in LM(tk-) cell.

We have observed that the peptide galanin -7-29, which

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binds selectively to GALR3 over GALR1 and to GALR2 over
GALR1, induces feeding in rats when injected i.c.v.
Another peptide, shown in binding and functional studies
to selectively bind to the GALR2 receptor over both GALR1

5 and GALR3, did not stimulate feeding when injected i.c.v.
Taken together, these results suggest a role for GALR3 in
mediating galanin-induced feeding.

Table 8.   Comparison of rank orders of $EC_{50}s$ for
10 stimulation of GIRKs, apparent binding affinities (K_i) and
$EC_{50}s$ for stimulation of feeding behavior *in vivo*.

Peptide	Oocyte EC_{50} (nM)	rat GALR3 Cos-7 K_i (nM)	human GALR3LM(tk-) Ki (nM)
M32	45	1.9	6.0
p-Galanin	222	5.1	
C7	343	23.0	8.1
human galanin	381		
gal -7 to 29	1,906	3.3	28.8
gal 1-16	2,030	51.9	319
M40	2,265	103.0	281

<u>Further pharmacologic characterization galaninergic peptides</u>

Peptide ligands were evaluated in binding and functional assays (Tables 9-11).

Table 9. Binding Data for Rat Galanin Receptors

Peptide	Ki (nM) from porcine 125I-galanin binding assay		
	Rat GalR1 CHO	Rat GALR2 LMTK #4	Rat GALR3 293 #105
(3-iodo-L-Tyr9)-(3-iodo-L-Tyr26)-galanin			
M32	0.70	0.69	1.32
C7	1.44	0.56	11.75
Rat galanin 1-29	0.31	1.43	2.73
porcine galanin	0.32	1.02	2.81
M35	0.37	4.27	3.24
(-7) to (+29) galanin, porcine	36.31	3.16	21.38
galantide	0.67	2.14	11.48
(-)9 to (+)29 galanin, porcine	51.29	3.47	4.17
Human galanin	0.62	2.54	53.09
Tyr9-iodo-M35			
M40	7.76	3.76	85.11
Porcine galanin 1-12			

Peptide	Ki (nM) from porcine 125I-galanin binding assay		
	Rat GalR1 CHO	Rat GALR2 LMTK #4	Rat GALR3 293 #105
Porcine galanin 1-15			
porcine galanin 1-16	2.45	2.75	138.04
D-Trp2-(d-iodo-L-Tyr9)-(3-iodo-L-Tyr26)-galanin	> 1000	1.51	181.97

Table 10: Binding Data for Human Receptors

Peptide	Ki (nM) from porcine 125I-galanin binding assay		
	Hum GALR1 LM(tk-)	Hum GALR2 CHO	Hum GALR3 LMTK- #8
(3-iodo-L-Tyr9)-(3-iodo-L-Tyr26)-galanin	0.21	0.40	1.43
M32	0.26	1.45	6.03
C7	0.26	0.63	8.13
Rat galanin 1-29	0.29	1.62	8.81
porcine galanin	0.23	0.97	8.97
M35	0.11	1.95	14.62
(-7) to (+29) galanin, porcine	6.84	4.95	28.84
galantide	0.25	1.08	40.18

Peptide	Ki (nM) from porcine 125I-galanin binding assay		
	Hum GALR1 LM(tk-)	Hum GALR2 CHO	Hum GALR3 LMTK- #8
(-)9 to (+)29 galanin, porcine	7.85	5.43	50.12
Human galanin	0.44	2.34	69.41
Tyr9-iodo-M35	0.83	1.45	87.10
M40	2.38	4.04	280.54
Porcine galanin 1-12	61.66	5.17	306.67
Porcine galanin 1-15	3.98	6.13	309.03
porcine galanin 1-16	1.89	5.37	319.15
D-Trp2-(d-iodo-L-Tyr9)-(3-iodo-L-Tyr26)-galanin	169.82	21.38	933.25
Porcine galanin 3-29	> 1000	> 1000	> 1000
Porcine galanin 1-9	> 1000	> 1000	> 1000
human galanin 3-30	> 1000	> 1000	> 1000
porcine galanin 1-13	7.94	28.18	> 1000
GMAP 44-59 amide	> 1000	> 1000	> 1000
GMAP 25-41 amide	> 1000	> 1000	> 1000
GMAP 16-41 amide	> 1000	> 1000	> 1000

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Peptide	Ki (nM) from porcine 125I-galanin binding assay		
	Hum GALR1 LM(tk-)	Hum GALR2 CHO	Hum GALR3 LMTK- #8
GMAP 1-41 amide	> 1000	> 1000	> 1000

Table 11. Functional Data at Galanin Receptors

Peptide	EC$_{50}$ (nM)		
	Rat GALR1 LM(tk-) cAMP	Rat GALR2 CHO #79 AA	Human GALR3 GIRK
p gal 1-16	0.34	2.63	2000
p galanin	**0.06**	**1.25**	**238**
human galanin	0.21	0.74	7340
C7	0.52	2.41	343
M40	0.82	2.69	5030
M32	0.34	2.51	45
rat gal	0.06	0.71	

Experimental Discussion

Using a combination of homology and expression cloning strategies, nucleic acids have been isolated encoding a novel galanin receptor, termed GALR3, that is distinct from the previously cloned GALR1 and GALR2 receptors.

The rat GALR3 gene, whose sequence is derived from cDNA, does not have any other MET upstream of the proposed starting MET, in any of the three possible reading frames.

The human GALR3 gene contains two in-frame METs: the first (as one reads 5' to 3') will be referred to herein as the "upstream MET" and the second (i.e., closer to TM1) will be referred to herein as the "downstream MET." Both the upstream and downstream METs are shown in Figure 4 (Seq. ID No. 4). Based on data currently available, it is believed that the downstream MET is likely to be the correct initiating methionine. It is theoretically possible that the upstream MET might be the initiating MET. It is to be understood that the present invention includes both the receptor beginning at the downstream MET and the receptor beginning at the upstream MET.

The existence of multiple galanin receptor subtypes suggests the potential for the design and discovery of novel subtype selective compounds. In this regard, the expression of the cDNA encoding the GALR3 receptor in cultured cell lines and other cells provides a unique tool for the discovery of therapeutic agents targeted at galanin receptors.

The localization of GALR1 receptors to multiple brain regions (Gustafson, et al., 1996; Parker, et al., 1995) and the identification of GALR3 in a hypothalamic cDNA

library, suggests multiple therapeutic indications for the use of galanin receptor-selective drugs. These include feeding, cognition, analgesia and/or sensory processing, and anxiety and depression.

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The observation that galanin is co-released with norepinephrine from sympathetic nerve terminals suggests that galanin could act via galanin receptors in the periphery to modulate nearly every physiological process

10   controlled by sympathetic innervation. Additional therapeutic indications not directly related to localization include diabetes, hypertension, cardiovascular disorders, regulation of growth hormone release, regulation of fertility, gastric ulcers,

15   gastrointestinal motility/transit/absorption/secretion, glaucoma, inflammation, immune disorders, respiratory disorders (e.g., asthma, emphysema).

The physiological and anatomical distribution of galanin-

20   containing neurons suggests potential roles of galanin receptors mediating effects on cognition, analgesia, neuroendocrine regulation, control of insulin release and control of feeding behavior. Of particular relevance to the role of the novel GALR3 receptor, are those functions

25   mediated by galanin receptors in the rat hypothalamus.

Studies in rats indicate that the injection of galanin in the hypothalamus increases food intake (Kyrouli et al, 1990, and Schick et al, 1993) and that this stimulatory

30   effect of galanin is blocked by prior administration of M40 and C7 (Liebowitz and Kim, 1992; and Corwin, 1993). The expression of the mRNA encoding the GALR1 receptor in the rat hypothalamus (Parker et al., 1995; Gustafson et al., 1996), and the fact that the novel GALR3 receptor

35   was identified in a cDNA library prepared from rat

hypothalamus argues in favor of the involvement of one or more galanin receptor subtypes in the regulation of feeding behavior. However, the original evidence against the involvement of GALR1 in the stimulation of feeding behavior stems from the fact that M40 and C7 are known to be agonists, and not antagonists, in cell lines expressing human and rat GALR1 receptors (Heuillet et al. 1994; Hale et al. 1993; and Bartfai et al. 1993).

Peptide displacement assays indicate that the rat GALR3 receptor has a unique pharmacological profile. The low affinity for M40, in particular, invites further speculation as to the physiological role of the rat GALR3 receptor. It is noted that M40 was reported to be inactive, for example, when tested for antagonism of galaninergic inhibition of glucose-stimulated insulin release in rat pancreas, (Bartfai, 1993). In another example, intrathecal M40 was a weak antagonist of the galanin-facilitated flexor reflex in rat (Xu, 1995). It was observed in feeding assays that M40 was less potent but as effective as galanin in stimulating food intake when injected i.c.v. into rat brain. The data are consistent with a role for the GALR3 receptor in a range of physiologic and pathophysiologic functions including diabetes, pain, obesity and eating disorders, and furthermore suggest that the rat GALR3 receptor may represent a target for the design of therapeutic compounds. The cloning of the rat GALR3 receptor further enables the design and development of in vitro functional assays to determine the agonist or antagonist properties of peptides and drug development candidates.

References

Ahrén, B. and S. Lindskog (1992) *Int. J. Pancreatol.*
11:147-160.

Amiranoff, B. A.M. Lorinet, and M. Laburthe (1991) *Eur.
J. Biochem.* 195:459-463.

Amiranoff, B. A.L. Servin, C. Rouyer-Fessard, A.
Couvineau, K. Tatemoto, and M. Laburthe (1987) *Endocrin.*
121:284-289.

Aruffo, A. and B. Seed (1987) *Proc. Natl. Acad. Sci. USA*
84:8573-8577.

Bhathena, S.J., H.K. Oie, A.F. Gazdar, N.R.Voyles, S.D.
Wilkins, and L. Recant (1982) *Diabetes* 31:521-531.

Bartfai,T., K. Bedecs, T. Land, Ü. Langel, R.
Bertorelli, P. Girotti, S. Consolo, Y.-J. Yu, Z.
Weisenfeld-Hallin, S. Nilsson, V. Pieribone, and T.
Hökfelt (1991) *Proc. Natl. Acad. Sci. USA* 88:10961-10965.

Bartfai,T., T. Hokfelt, and U. Langel, *Crit. Rev.
Neurobiol. (1993)* 7:229-274.

Bartfai,T., Ü. Langel, K. Bedecs, S. Andell, T. Land, S.
Gregersen, B. Ahren, P. Girotti, S. Consolo, R. Corwin,
J. Crawley, X. Xu, Z. Weisenfeld-Hallin, and T. Hökfelt
(1993) *Proc. Natl. Acad. Sci. USA* 88:11287-11291.

Bennet, W.M., S.F. Hill, M.A. Ghatei, and S.R. Bloom

- 146 -

(1991) *J. Endocrin.* 130:463-467.

Boyle, M.R., C. B. Verchere, G. McKnight, S. Mathews, K. Walker, and G.J. Taborsky, Jr. (1994) *Reg. Peptides* 50:1-11.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. <u>Anal. Biochem</u>. 72: 248-254.

Burbach, J.P. and O.C. Meijer (1992) *Eur. J. Pharmacol.* 227:1-18.

Burgevin, M.-C., Loquet, I., Quarteronet, D., and Habert-Ortoli, E. (1995) *J. Molec. Neurosci.*, 6:33-41.

Bush, A.W., Borden, L.A., Greene, L.A., and Maxfield, F.R. (1991) *J. Neurochem.* 57:562-574.

Chan-Palay, V. (1988) *J.Comp.Neurol.* 273:543-557.

Chen, Y., A. Fournier, A. Couvineau, M. Laburthe, and B. Amiranoff (1993) *Proc. Natl. Acad. Sci. USA* 90:3845-3849.

Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. (1979) *Biochemistry* 18:5294-5299.

Consolo, S., R. Bertorelli, P. Girotti, C. La Porta, T. Bartfai, M. Parenti, and M. Zambelli (1991) *Neurosci. Lett.* 126:29-32.

- 147 -

Crawley, J.N. (1993) *Behav. Brain Res.* 57:133-141.

Crawley, J.N., J.K. Robinson, Ü. Langel, and T. Bartfai (1993) *Brain. Res.* **600**:268-272.

5

Cullen, B. (1987).   Use of eurkaryotic expression technology in the functional analysis of cloned genes. Methods Enzymol. 152: 685-704.

10   D'Andrea, A.D., H.F. Lodish, and G.W. Gordon (1989) *Cell* 57:277-285.

Dascal, N., W. Schreibmayer, N. F. Lim, W. Wang, C. Chavkin, L. DiMagno, C. Labarca, B. L. Kieffer, C.
15   Gaveriaux-Ruff, D. Trollinger, H. A. Lester, and N. Davidson (1993) *Proc. Natl. Acad. Sci. USA* 90:10235-10239.

Fisone, G., C.F. Wu, S. Consolo, Ö. Nördstrom, N. Brynne,
20   T.   Bartfai,   T.   Melander,   T.   Hökfelt (1987) *Proc. Natl. Acad. Sci USA* 84:7339.

Gearing, D.P., King, J.A.,Gough, N.M. and Nicola N.A. (1989) *EMBO J.* 8:3667-3676.

25

Gerald, C., M. Walker, T. Branchek, and R. Weinshank (1994) DNA Encoding a Human Neuropeptide Y/Peptide YY (Y2) Receptor and Uses Thereof, United States Patent No. 5,545,549, issued August 13, 1996, U.S. Serial No.
30   08/192,288, filed February 3, 1994.

Gillison, S.L., and W.G. Sharp (1994) *Diabetes* 43:24-32.

- 148 -

Gregersen, S., S. Lindskog, T. Land, U. Langel, T. Bartfai, and B. Ahren (1993) *Eur J. Pharmacol.* 232:35-39.

Gu, Z.-F., W.J. Rossowski, D.H. Coy, T.K. Pradhan, and R.T. Jensen (1993) *J. Phamacol. Exper. Ther.* 266:912-918.

Gu, Z.-F., Pradhan, T.K., Coy, D.H., and Jensen, R.T. (1995) *J. Pharmacol. Exp. Ther.*, 272:371-378.

Gubler, U abd B.J. Hoffman. (1983). A simple and very efficient method for generating cDNA libraries. Gene 25: 263-269.

Gundersen, C. B., R. Miledi, and I. Parker (1983) *Proc. R. Soc. London Ser. B* 219:103-109.

Gustafson, E.L., Smith, K.E., Durkin, M.M., Gerald, C., and Branchek, T.A. (1996) *Neuroreport*, 7:953-957.

Habert-Ortoli, E., Amiranoff, B., Loquet, I., Laburthe, M., and J.-F. Mayaux (1994) *Proc. Natl. Acad. Sci.USA* 91:9780-9783.

Hedlund, P.B., N. Yanaihara, and K. Fuxe (1992) *Eur. J. Pharm.* 224:203-205.

Heuillet, E., Bouaiche, Z., Menager, J., Dugay, P., Munoz, N., Dubois, H., Amiranoff, B., Crespo, A., Lavayre, J., Blanchard, J.-C., and Doble, A. (1994) *Eur. J. Pharmacol.*, 269:139-147.

- 149 -

Kaplan, L.M., S.M. Gabriel, J.I. Koenig, M.E. Sunday, E.R. Spindel, J.B. Martin, and W.W. Chin (1988) *Proc. Natl. Acad. Sci.USA* 85:7408-7412.

Kieffer, B., Befort, K., Gaveriaux-Ruff, C. and Hirth, C.G. (1992). The δ-opioid receptor: Isolation of a cDNA by expression cloning and . Proc. Natl. Acad. Sci. USA 89:12048-12052.

Kluxen, F.W., Bruns, C. and Lubbert H. (1992). Expression cloning of a rat brain somatostatin receptor cDNA. Proc. Natl. Acad. Sci. USA 89:4618-4622.

Kofuji P., Davidson N., Lester, H.A. (1995). Evidence that neuronal G-protein-gated inwardly rectifying K+ channels are activated by G beta gamma subunits and function as heteromultimers. *Proc. Natl. Acad. Sci. USA* 92:6542-6546.

Kornfeld, R. and Kornfeld, S. (1985). Assembly of asparagine linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.

Kozak, M. (1989). The scanning model for translation: an update. J. Cell Biol. 108: 229-241.

Kozak, M. (1991). Structural features in eukaryotic mRNAs that modulate the initiation of translation. J. Biol. Chem. 266: 19867-19870.

Krapivinsky, G., E. A. Gordon, K. Wickman, B. Velimirovic, L. Krapivinsky, and D. E. Clapham (1995) *Nature* 374:135-141.

Krapivinsky G., Krapivinsky L., Velimirovic B., Wickman K., Navarro B., Clapham D.E. (1995b). The cardiac inward rectifier K+ channel subunit, CIR, does not comprise the ATP-sensitive K+ channel, IKATP. *J. Biol. Chem.* 270:28777-28779.

Kubo, Y., E. Reuveny, P. A. Slesinger, Y. N. Jan, and L. Y. Jan (1993) *Nature* 364:802-806.

Kyrkouli, S.E., B.G. Stanley, R.D. Seirafi and S.F. Leibowitz (1990) *Peptides* 11:995-1001.

Lagny-Pourmir, I., A.M. Lorinet, N. Yanaihara, and M. Laburthe (1989) *Peptides* 10:757-761.

Landschultz, W.H., Johnson, P.F. and S.L. McKnight. (1988). The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. <u>Science</u> 240: 1759-1764.

Lazareno, S. and N. Birdsall. (1993). Pharmacological characterization of acetylcholine-stimulated [35S]-GTPγS binding mediated by human muscarinic m1-m4 receptors: antagonist studies. <u>Br. J. Pharmacol.</u> 109:1120-1127.

Leibowitz, S.F. and T. Kim (1992) *Brain Res.* 599:148-152.

Liman, E.R., Tytgat, J. and P. Hess (1992) Neuron 9:861-871.

Maggio, R., Vogel Z. and J. Wess. (1993). Coexpression studies with mutant muscarinic/adrenergic receptors

provide evidence for intermolecular "cross-talk" between G-protein-linked receptors. <u>Proc. Natl. Acad. Sci. USA</u> 90: 3103-3107.

5   McCormick, M. (1987). Sib Selection. <u>Methods in Enzymology</u>, **151**: 445-449.

Melander, T., C. Köhler, S. Nilsson, T. Hökfelt, E. Brodin, E. Theodorsson, and T. Bartfai (1988) *J. Chem.*
10   *Neuroanat.* 1:213-233.

Merchenthaler, I., F.J. López, and A. Negro-Vilar (1993) *Prog. Neurobiol.* **40**:711-769.

15   Miller, J. and Germain, R.N. (1986). Efficient cell surface expression of class II MHC molecules in the absence of associated invariant chain. <u>J. Exp. Med.</u> **164**: 1478-1489.

20   North R.A. (1989). Drug receptors and the inhibition of nerve cells. *Br. J.Pharmacol.* 98:13-23.

Nowak MW, Kearney PC, Sampson JR, Saks ME, Labarca CG, Silverman SK, Zhong W, Thorson J, Abelson JN, Davidson N
25   (1995) Nicotinic receptor binding site probed with unnatural amino acid incorporation in intact cells. <u>Science</u> 268:439-442.

Ögren, S.-O., T. Hökfelt, K. Kask, Ü. Langel, and T.
30   Bartfai (1992) *Neurosci.* 51:1.

Palazzi, E., G. Fisone, T. Hökfelt, T. Bartfai, and S.

- 152 -

Consolo (1988) *Eur. J. Pharmacol.* **148**:479.

Parker, E.M., Izzarelli, D.G., Nowak, H.P., Mahle, C.D., Iben, L.G., Wang, J., and Goldstein, M.E. (1995) *Mol. Brain Res.*, **34**:179-189.

Post, C., L. Alari, and T. Hökfelt (1988) *Acta Physiol. Scand.* **132**:583.

Probst, W.C., Snyder, L.A., Schuster, D.I., Brosius, J and Sealfon, S.C. (1992). Sequence alignment of the G-protein coupled receptor superfamily. *DNA and Cell Bio.* **11**: 1-20.

Quick, M. W. and H. A. Lester. (1994) *Meth. Neurosci.* **19**:261-279.

Sambrook, J., Fritsch, E.F., and T. Maniatis (1989) Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sanger, S. (1977) *Proc. Natl. Acad. Sci. USA* **74**:5463-5467.

Servin, A.L., B. Amiranoff, C. Rouyer-Fessard, K. Tatemoto, and M. Laburthe (1987) *Biochem. Biophys. Res. Comm.* **144**:298-306.

Shen,Y., Monsma, F.J. Jr., Metcalf, M.A., Jose, P.A., Hamblin, M.W., and Sibley, D.R. (1993) Molecular Cloning and Expression of a 5-Hydroxytryptamine, Serotonin

- 153 -

Receptor Subtype. *J. Biol. Chem.* 268:18200-18204.

Sims, J.E., C.J. March, D. Cosman, M.B. Widmer, H.R. Macdonald, C.J. McMahan, C.E. Grubin, J.M. Wignal, J.L. Jackson, S.M. Call, D. Freind, A.R. Alpert, S. Gillis, D.L. Urdal, and S.K. Dower (1988) *Science* 241:585-588.

Skofitsch, G. and D.M. Jacobowitz (1985) *Peptides* 6:509-546.

Skofitsch, G., M.A. Sills, and D.M. Jacobowitz (1986) *Peptides* 7:1029-1042.

Smith, K.E., L.A. Borden, P.R. Hartig, T. Branchek, and R.L. Weinshank (1992) *Neuron* 8: 927-935.

Smith, K.E., L.A. Borden, C-H.D. Wang, P.R. Hartig, T.A. Branchek, and R.L. Weinshank (1992a) *Mol. Pharmacol.* 42:563-569.

Smith, K.E., S.G. Fried, M.M. Durkin, E.L. Gustafson, L.A. Borden, T.A. Branchek, and R.L. Weinshank (1995) *FEBS Letters*, 357:86-92.

Stühmer, W. (1992) Meth. Enzymology 207:319-339.

Sundström, E., T. Archer, T. Melander, and T. Hökfelt (1988) *Neurosci. Lett.* 88:331.

Takahashi, T., E. Neher, and B. Sakmann (1987) *Proc. Natl. Acad. Sci. USA* 84:5063-6067.

- 154 -

Tempel, D.L., K.J. Leibowitz, and S.F. Leibowitz (1988) *Peptides* 9:300-314.

Tian, W.-N., Duzic, E., Lanier, S. and R.C. Deth. (1994). Determinants of α2-adrenergic receptor activation of G proteins: evidence for a precoupled receptor/G protein state. <u>Mol. Pharm.</u> 45:524-531.

Vrontakis, M.E., L.M. Peden, M.L Duckworth, and H.G. Friesen (1987) *J. Biol. Chem.* 262:16755-16760.

Warden, D. and H.V. Thorne. (1968). Infectivity of polyoma virus DNA for mouse embryo cells in presence of diethylaminoethyl-dextran. <u>J. Gen. Virol.</u> 3: 371.

Wiesenfeld-Hallin, Z., X.J. Xu, J.X. Hao, and T. Hökfelt (1993) *Acta Physiol.Scand.* 147:457-458.

Wiesenfeld-Hallin, Z., et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:3334-3337.

Wynick D., D.M. Smith, M. Ghatei, K. Akinsanya, R. Bhogal, P. Purkiss, P. Byfield, N. Yanaihara, and S.R. Bloom (1993) *Proc. Natl. Acad. Sci. USA* 90:4231-4245.0

Xu, X.-J., Wuesebfeld-Hallin, Z., Langel, U., Bedecs, K. and Bartfai, T. (1995). New high affinity peptide antagonists to the spinal galanin receptor. *Br. J. Pharmacol.* 116:2076-2080.

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What is claimed is:

1.   An isolated nucleic acid encoding a GALR3 receptor.

2.   The nucleic acid of claim 1, wherein the nucleic acid is DNA.

3.   The DNA of claim 2, wherein the DNA is cDNA.

4.   The DNA of claim 2, wherein the DNA is genomic DNA.

5.   The nucleic acid of claim 1, wherein the nucleic acid is RNA.

6.   The nucleic acid of claim 1, wherein the nucleic acid encodes a vertebrate GALR3 receptor.

7.   The nucleic acid of claim 1, wherein the nucleic acid encodes a mammalian GALR3 receptor.

8.   The nucleic acid of claim 1, wherein the nucleic acid encodes a rat GALR3 receptor.

9.   The nucleic acid of claim 1, wherein the nucleic acid encodes a human GALR3 receptor.

10.   The nucleic acid of claim 7, wherein the nucleic acid encodes a receptor characterized by an amino acid sequence in the transmembrane region which has a homology of 70% or higher to the amino acid sequence in the transmembrane region of the rat

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GALR3 receptor and a homology of less than 70% to
the amino acid sequence in the transmembrane region
of any GALR1 receptor.

5   11.   The nucleic acid of claim 7, wherein the nucleic
           acid encodes a mammalian GALR3 receptor which has
           substantially the same amino acid sequence as does
           the GALR3 receptor encoded by the plasmid K1086
           (ATCC Accession No. 97747).

10

     12.   The nucleic acid of claim 8, wherein the nucleic
           acid encodes a rat GALR3 receptor which has an amino
           acid sequence encoded by the plasmid K1086 (ATCC
           Accession No. 97747).

15

     13.   The nucleic acid of claim 8, wherein the nucleic
           acid encodes a rat GALR3 receptor having
           substantially the same amino acid sequence as the
           amino acid sequence shown in Figure 2 (Seq. ID No.
20   2).

     14.   The nucleic acid of claim 8, wherein the nucleic
           acid encodes a rat GALR3 receptor having the amino
           acid sequence shown in Figure 2 (Seq. ID No. 2).

25

     15.   The nucleic acid of claim 7, wherein the nucleic
           acid encodes a mammalian galanin receptor which has
           substantially the same amino acid sequence as does
           the GALR3 receptor encoded by the plasmid pEXJ-
30   hGalR3 (ATCC Accession No. 97827).

     16.   The nucleic acid of claim 9, wherein the nucleic
           acid encodes a human galanin receptor which has an
           amino acid sequence encoded by the plasmid pEXJ-

hGalR3 (ATCC Accession No. 97827).

17. The nucleic acid of claim 9, wherein the human GALR3
    receptor has a sequence, which sequence comprises
5   substantially the same amino acid sequence as the
    sequence shown in Figure 4 (Seq. I.D. No. 4) from
    amino acid 60 through amino acid 427.

18. The nucleic acid of claim 17, wherein the human
10 GALR3 receptor has a sequence, which sequence
    comprises the sequence shown in Figure 4 (Seq. I.D.
    No. 4) from amino acid 60 through amino acid 427.

19. An isolated nucleic acid encoding a modified GALR3
15 receptor, which differs from a GALR3 receptor by
    having an amino acid(s) deletion, replacement or
    addition in the third intracellular domain.

20. The nucleic acid of claim 19, wherein the modified
20 GALR3 receptor differs by having a deletion in the
    third intracellular domain.

21. The nucleic acid of claim 19, wherein the modified
    GALR3 receptor differs by having a replacement or
25 addition to the third intracellular domain.

22. A purified GALR3 receptor protein.

23. A vector comprising the nucleic acid of claim 1.

30

24. A vector comprising the nucleic acid of claim 9.

25. A vector of claim 23 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding a GALR3 receptor as to permit expression thereof.

26. A vector of claim 23 adapted for expression in an amphibian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the amphibian cell operatively linked to the nucleic acid encoding a GALR3 receptor as to permit expression thereof.

27. A vector of claim 23 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a GALR3 receptor as to permit expression thereof.

28. A vector of claim 23 adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the GALR3 receptor as to permit expression thereof.

29. A vector of claim 28 which is a baculovirus.

30. A vector of claim 23 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the

nucleic acid encoding a GALR3 receptor as to permit expression thereof.

31. A vector of claim 23 wherein the vector is a plasmid.

32. The plasmid of claim 31 designated K1086 (ATCC Accession No. 97747).

33. The plasmid of claim 31 designated pEXJ-hGalR3 (ATCC Accession No. 97827).

34. The plasmid of claim 31 designated pEXJ-RGalR3T (ATCC Accession No. 97826).

35. A cell comprising the vector of claim 23.

36. A cell of claim 35, wherein the cell is a non-mammalian cell.

37. A cell of claim 36, wherein the non-mammalian cell is a Xenopus oocyte cell or a Xenopus melanophore cell.

38. A cell of claim 35, wherein the cell is a mammalian cell.

39. A mammalian cell of claim 38, wherein the cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a mouse Y1 cell, a LM(tk-) cell or a CHO cell.

40.   The 293 human embryonic kidney cell of claim 39
      designated 293-rGalR3-105 (ATCC Accession No. CRL-
      12287).

5   41.   The LM(tk-) cell of claim 39 designated L-hGalR3-228
          (ATCC Accession No. CRL-12373).

42.   An insect cell comprising the vector of claim 28.

10   43.   An insect cell of claim 42, wherein the insect cell
           is an Sf9 cell.

44.   An insect cell of claim 42, wherein the insect cell
      is an Sf21 cell.

15

45.   A membrane preparation isolated from the cell of
      claim 35 or 42.

46.   A nucleic acid probe comprising at least 15
20   nucleotides, which probe specifically hybridizes
          with a nucleic acid encoding a GALR3 receptor,
          wherein the probe has a unique sequence
          corresponding to a sequence present within one of
          the two strands of the nucleic acid encoding the
25   GALR3 receptor contained in plasmid K1086.

47.   A nucleic acid probe comprising at least 15
          nucleotides, which probe specifically hybridizes
          with a nucleic acid encoding a GALR3 receptor,
30   wherein the probe has a unique sequence
          corresponding to a sequence present within (a) the
          nucleic acid sequence shown in Figure 1 (Seq. ID No.
          1) or (b) the reverse complement to the nucleic acid

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sequence shown in Figure 1 (Seq. ID No. 1).

48. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR3 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding the GALR3 receptor contained in plasmid pEXJ-hGalR3.

49. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR3 receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figure 3 (Seq. ID No. 3) or (b) the reverse complement to the nucleic acid sequence shown in Figure 3 (Seq. ID No. 3).

50. The nucleic acid probe of claim 48 or 49, wherein the nucleic acid is DNA.

51. The nucleic acid probe of claim 48 or 49, wherein the nucleic acid is RNA.

52. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a unique fragment of the sequence of a nucleic acid molecule encoding a GALR3 receptor.

53. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to the antisense sequence of a unique

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fragment of the sequence of a nucleic acid molecule
encoding a GALR3 receptor.

54. An antisense oligonucleotide having a sequence
capable of specifically hybridizing to the RNA of
claim 5, so as to prevent translation of the RNA.

55. An antisense oligonucleotide having a sequence
capable of specifically hybridizing to the genomic
DNA of claim 4.

56. An antisense oligonucleotide of either of claims 54
or 55, wherein the oligonucleotide comprises
chemically modified nucleotides or nucleotide
analogues.

57. An antibody capable of binding to a GALR3 receptor
encoded by the nucleic acid of claim 1.

58. The antibody of claim 57, wherein the GALR3 receptor
is a human GALR3 receptor.

59. An antibody capable of competitively inhibiting the
binding of the antibody of claim 57 to a GALR3
receptor.

60. An antibody of claim 57, wherein the antibody is a
monoclonal antibody.

61. A monoclonal antibody of claim 60 directed to an
epitope of a GALR3 receptor present on the surface
of a GALR3 receptor expressing cell.

62. A pharmaceutical composition comprising an amount of the oligonucleotide of claim 54 capable of passing through a cell membrane effective to reduce expression of a GALR3 receptor and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

63. A pharmaceutical composition of claim 62, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

64. A pharmaceutical composition of claim 63, wherein the substance which inactivates mRNA is a ribozyme.

65. A pharmaceutical composition of claim 62, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by the cells after binding to the structure.

66. A pharmaceutical composition of claim 65 wherein the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

67. A pharmaceutical composition which comprises an amount of the antibody of claim 57 effective to block binding of a ligand to the GALR3 receptor and a pharmaceutically acceptable carrier.

68. A transgenic nonhuman mammal expressing DNA encoding a GALR3 receptor of claim 1.

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69.   A transgenic nonhuman mammal comprising a homologous
       recombination knockout of the native GALR3 receptor.

70.   A transgenic nonhuman mammal whose genome comprises
  5   antisense DNA complementary to DNA encoding a GALR3
       receptor of claim 1 so placed as to be transcribed
       into antisense mRNA which is complementary to mRNA
       encoding a GALR3 receptor and which hybridizes to
       mRNA encoding a GALR3 receptor, thereby reducing its
 10   translation.

 71.   The transgenic nonhuman mammal of either of claims
       68 or 69, wherein the DNA encoding a GALR3 receptor
       additionally comprises an inducible promoter.

 15

 72.   The transgenic nonhuman mammal of either of claims
       68 or 69, wherein the DNA encoding a GALR3 receptor
       additionally comprises tissue specific regulatory
       elements.

 20

 73.   A transgenic nonhuman mammal of any one of claims
       68, 69 or 70, wherein the transgenic nonhuman mammal
       is a mouse.

 25   74. A process for identifying a chemical compound which
       specifically binds to a GALR3 receptor which
       comprises contacting cells containing DNA encoding
       and expressing on their cell surface the GALR3
       receptor, wherein such cells do not normally express
 30   the GALR3 receptor, with the compound under
       conditions suitable for binding, and detecting
       specific binding of the chemical compound to the
       GALR3 receptor.

75. A process for identifying a chemical compound which specifically binds to a GALR3 receptor which comprises contacting a membrane fraction from a cell extract of cells containing DNA encoding and

5   expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the GALR3 receptor.

10

76. The process of claim 74 or 75, wherein the GALR3 receptor is a mammalian GALR3 receptor.

77. The process of claim 74 or 75, wherein the GALR3

15   receptor has substantially the same amino acid sequence as encoded by the plasmid K1086 (ATCC Accession No. 97747).

78. The process of claim 74 or 75, wherein the GALR3

20   receptor has substantially the same sequence as the amino acid sequence shown in Figure 2 (Seq. ID No. 2).

79. The process of claim 74 or 75, wherein the GALR3

25   receptor has the amino acid sequence shown in Figure 2 (Seq. ID No. 2).

80. The process of claims 74 or 75, wherein the GALR3 receptor has substantially the same amino acid

30   sequence as encoded by the plasmid pEXJ-hGalR3 (ATTC Accession No. 97827).

81. The process of claim 74 or 75, wherein the GALR3 receptor has a sequence, which sequence comprises

substantially the same amino acid sequence as a
sequence shown in Figure 4 (Seq. ID No. 4) from
amino acid 60 through amino acid 427.

5   82.   The process of claim 74 or 75, wherein the GALR3
              receptor has a sequence, which sequence comprises a
              sequence shown in Figure 4 (Seq. ID No. 4) from
              amino acid 60 through amino acid 427.

10   83.   The process of claim 74 or 75, wherein the cells are
              transfected   with   plasmid   pEXJ-RGalR3T   (ATCC
              Accession No. 97826).

       84.   The process of claim 81, wherein the compound is not
15   previously known to bind to a GALR3 receptor.

       85.   A compound determined by the process of claim 84.

       86.   A process of claim 81, wherein the cell is an insect
20   cell.

       87.   A process of claim 81, wherein the cell is a
              mammalian cell.

25   88.   A process of claim 87, wherein the cell is
              nonneuronal in origin.

       89.   A process of claim 88, wherein the nonneuronal cell
              is a COS-7 cell, 293 human embryonic kidney cell, a
30   CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-)
              cell.

90.   A process of claim 87, wherein the compound is not previously known to bind to a GALR3 receptor.

91.   A compound determined by the process of claim 90.

5

92.   A process involving competitive binding for identifying a chemical compound which specifically binds to a GALR3 receptor which comprises separately contacting cells expressing on their cell surface

10   the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable

15   for binding of both compounds, and detecting specific binding of the chemical compound to the GALR3 receptor, a decrease in the binding of the second chemical compound to the GALR3 receptor in the presence of the chemical compound indicating

20   that the chemical compound binds to the GALR3 receptor.

93.   A process involving competitive binding for identifying a chemical compound which specifically

25   binds to a human GALR3 receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with both

30   the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the

35   GALR3 receptor, a decrease in the binding of the

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second chemical compound to the GALR3 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GALR3 receptor.

94.   A process of claim 92 or 93, wherein the GALR3 receptor is a mammalian GALR3 receptor.

95.   The process of claim 94, wherein the GALR3 receptor has substantially the same amino acid sequence as encoded by plasmid K1086 (ATCC Accession No. 97747).

96.   The process of claim 92 or 93, wherein the GALR3 receptor has substantially the same amino acid sequence as shown in Figure 2 (Seq. ID No. 2).

97.   The process of either of claims 92 or 93, wherein the GALR3 receptor has the amino acid sequence shown in Figure 2 (Seq. ID No. 2).

98.   The process of claim 92 or 93, wherein the GALR3 receptor has substantially the same amino acid sequence as encoded by plasmid pEXJ-hGalR3 (ATCC Accession No. 97827).

99.   The process of claim 92 or 93, wherein the GALR3 receptor has a sequence, which sequence comprises substantially the same amino acid sequence as the sequence shown in Figure 4 (Seq. ID No. 4) from amino acid 60 through amino acid 427.

100. The process of claim 92 or 93, wherein the GALR3 receptor has a sequence, which sequence comprises a

sequence shown in Figure 4 (Seq. ID No. 4) from amino acid 60 through amino acid 427.

101. The process of claim 92 or 93, wherein the cells are transfected with plasmid pEXJ-RGalR3T (ATCC Accession No. 97826).

102. The process of claim 100, wherein the cell is an insect cell.

103. The process of claim 100, wherein the cell is a mammalian cell.

104. The process of claim 103, wherein the cell is nonneuronal in origin.

105. The process of claim 103, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-) cell.

106. The process of claim 102, wherein the compound is not previously known to bind to a GALR3 receptor.

107. A compound determined by the process of claim 106.

108. A method of screening a plurality of chemical compounds not known to bind to a GALR3 receptor to identify a compound which specifically binds to the GALR3 receptor, which comprises

(a) contacting cells transfected with and

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expressing DNA encoding the GALR3 receptor with a compound known to bind specifically to the GALR3 receptor;

5   (b)   contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the GALR3 receptor, under conditions permitting binding of compounds known to bind the GALR3 receptor;

10

(c)   determining whether the binding of the compound known to bind to the GALR3 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of

15   the plurality of compounds; and if so

(d)   separately determining the binding to the GALR3 receptor of each compound included in the plurality of compounds, so as to thereby

20   identify the compound which specifically binds to the GALR3 receptor.

109. A method of screening a plurality of chemical compounds not known to bind to a GALR3 receptor to

25   identify a compound which specifically binds to the GALR3 receptor, which comprises

(a)   preparing a cell extract from cells transfected with and expressing DNA encoding the GALR3

30   receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a compound known to bind specifically to the GALR3 receptor;

(b)   contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the GALR3 receptor, under conditions permitting binding of compounds known to bind the GALR3 receptor;

(c)   determining whether the binding of the compound known to bind to the GALR3 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(d)   separately determining the binding to the GALR3 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the GALR3 receptor.

110.   A method of either of claims 108 or 109, wherein the GALR3 receptor is a mammalian GALR3 receptor.

111.   A method of either of claims 108 or 109, wherein the cell is a mammalian cell.

112.   A method of claim 111, wherein the mammalian cell is non-neuronal in origin.

113.   The method of claim 112, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell or an NIH-3T3 cell.

114.   A method of detecting expression of a GALR3 receptor

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by detecting the presence of mRNA coding for the
GALR3 receptor which comprises obtaining total mRNA
from the cell and contacting the mRNA so obtained
with the nucleic acid probe of claim 48 under
5   hybridizing conditions, detecting the presence of
mRNA hybridized to the probe, and thereby detecting
the expression of the GALR3 receptor by the cell.

115.   A method of detecting the presence of a GALR3
10   receptor on the surface of a cell which comprises
contacting the cell with the antibody of claim 57
under conditions permitting binding of the antibody
to the receptor, detecting the presence of the
antibody bound to the cell, and thereby detecting
15   the presence of a GALR3 receptor on the surface of
the cell.

116.   A method of determining the physiological effects of
varying levels of activity of GALR3 receptors which
20   comprises producing a transgenic nonhuman mammal of
claim 71 whose levels of GALR3 receptor activity are
varied by use of an inducible promoter which
regulates GALR3 receptor expression.

25   117.   A method of determining the physiological effects of
varying levels of activity of GALR3 receptors which
comprises producing a panel of transgenic nonhuman
mammals of claim 71 each expressing a different
amount of GALR3 receptor.

30

118.   A method for identifying an antagonist capable of
alleviating an abnormality wherein the abnormality
is alleviated by decreasing the activity of a GALR3
receptor comprising administering a compound to the
35   transgenic nonhuman mammal of any one of claims 68,

71, 72, or 73, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overactivity of a GALR3 receptor, the alleviation of the abnormality identifying the compound as an antagonist.

119. An antagonist identified by the method of claim 118.

120. A pharmaceutical composition comprising an antagonist identified by the method of claim 119 and a pharmaceutically acceptable carrier.

121. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a GALR3 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 120, thereby treating the abnormality.

122. A method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GALR3 receptor comprising administering a compound to the transgenic nonhuman mammal of any one of claims 68, 71, 72, or 73, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal, the alleviation of the abnormality identifying the compound as an agonist.

123. An agonist identified by the method of claim 122.

124. A pharmaceutical composition comprising an agonist

identified by the method of claim 122 and a pharmaceutically acceptable carrier.

125. A method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GALR3 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 124, thereby treating the abnormality.

126. A method for diagnosing a predisposition to a disorder associated with the activity of a specific human GALR3 receptor allele which comprises:

    a.   obtaining DNA of subjects suffering from the disorder;

    b.   performing a restriction digest of the DNA with a panel of restriction enzymes;

    c.   electrophoretically separating the resulting DNA fragments on a sizing gel;

    d.   contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human GALR3 receptor and labelled with a detectable marker;

    e.   detecting labelled bands which have hybridized to the DNA encoding a human GALR3 receptor of claim 9 labelled with a detectable marker to

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create a unique band pattern specific to the
DNA of subjects suffering from the disorder;

f.   preparing DNA obtained for diagnosis by steps
      a-e; and

g.   comparing the unique band pattern specific to
      the DNA of subjects suffering from the disorder
      from step e and the DNA obtained for diagnosis
      from step f to determine whether the patterns
      are the same or different and to diagnose
      thereby predisposition to the disorder if the
      patterns are the same.

127. The method of claim 126, wherein a disorder
     associated with the activity of a specific human
     GALR3 receptor allele is diagnosed.

128. A method of preparing the purified GALR3 receptor of
     claim 22 which comprises:

a.   inducing cells to express GALR3 receptor;

b.   recovering the receptor from the induced cells;
      and

c.   purifying the receptor so recovered.

129. A method of preparing the purified GALR3 receptor of
     claim 22 which comprises:

a.   inserting nucleic acid encoding the GALR3

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receptor in a suitable vector;

    b.   introducing the resulting vector in a suitable host cell;

    c.   placing the resulting cell in suitable condition permitting the production of the isolated GALR3 receptor;

    d.   recovering the receptor produced by the resulting cell; and

    e.   purifying the receptor so recovered.

130. A method of modifying feeding behavior of a subject which comprises administering to the subject an amount of a compound which is a GALR3 receptor agonist or antagonist effective to increase or decrease the consumption of food by the subject so as to thereby modify feeding behavior of the subject.

131. The method of claim 130, wherein the compound is a GALR3 receptor antagonist and the amount is effective to decrease the consumption of food by the subject.

132. The method of either of claims 130 or 131, wherein the compound is administered in combination with food.

133. The method of claim 130, wherein the compound is a

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GALR3 receptor agonist and the amount is effective to increase the consumption of food by the subject.

134. The method of either of claims 130 or 131, wherein the compound is administered in combination with food.

135. The method of claim 130, wherein the subject is a vertebrate, a mammal, a human or a canine.

136. The method of claim 131 or 133, wherein the compound binds selectively to a GALR3 receptor.

137. The method of claim 136, wherein the compound binds to the GALR3 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR1 receptor.

138. The method of claim 136, wherein the compound binds to the GALR3 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR2 receptor.

139. A method for determining whether a compound is a GALR3 antagonist which comprises:

(a) administering to an animal a GALR3 agonist and measuring the amount of food intake in the animal;

(b) administering to a second animal both the GALR3 agonist and the compound, and measuring the

amount of food intake in the second animal; and

(c)   determining whether the amount of food intake is reduced in the presence of the compound relative to the amount of food intake in the absence of the compound, so as to thereby determine whether the compound is a GALR3 antagonist.

140.   A method of screening a plurality of compounds to identify a compound which is a GALR3 antagonist which comprises:

(a)   administering to an animal a GALR3 agonist and measuring the amount of food intake in the animal;

(b)   administering to a second animal the GALR3 agonist and at least one compound of the plurality of compounds and measuring the amount of food intake in the animal;

(c)   determining whether the amount of food intake is reduced in the presence of at least one compound of the plurality relative to the amount of food intake in the absence of at least one compound of the plurality, and if so;

(d)   separately determining whether each compound is a GALR3 antagonist according to the method of claim 118, so as to thereby identify a compound which is a GALR3 antagonist.

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141. The method of either of claims 139 or 140, wherein the GALR3 agonist is galanin.

142. The method of either of claims 139 or 140, wherein the animal is a non-human mammal.

143. The method of claim 142, wherein the mammal is a rodent.

144. A process of claim 74 or 75, which further comprises determining whether the compound selectively binds to the GALR3 receptor relative to another galanin receptor.

145. The process of claim 144, wherein the determination whether the compound selectively binds to the GALR3 receptor comprises:

   (a) determining the binding affinity of the compound for the GALR3 receptor and for such other galanin receptor; and

   (b) comparing the binding affinities so determined, the presence of a higher binding affinity for the GALR3 receptor than for such other galanin receptor indicating that the compound selectively binds to the GALR3 receptor.

146. A process of claim 144, wherein the other galanin receptor is a GALR1 receptor.

147. A process of claim 144, wherein the other galanin

receptor is a GALR2 receptor.

148. A method of decreasing feeding behavior of a subject which comprises administering a compound which is a GALR3 receptor antagonist and a compound which is a Y5 receptor antagonist, the amount of such antagonists being effective to decrease the feeding behavior of the subject.

149. The method of claim 148, wherein the GALR3 antagonist and the Y5 antagonist are administered in combination.

150. The method of claim 148, wherein the GALR3 antagonist and the Y5 antagonist are administered once.

151. The method of claim 148, wherein the GALR3 antagonist and the Y5 antagonist are administered separately.

152. The method of claim 151, wherein the GALR3 antagonist and the Y5 antagonist are administered once.

153. The method of claim 151, wherein the galanin receptor antagonist is administered for about 1 week to 2 weeks.

154. The method of claim 151, wherein the Y5 receptor antagonist is administered for about 1 week to 2 weeks.

155. The method of claim 151, wherein the GALR3 antagonist and the Y5 antagonist are administered alternately.

5   156. The method of claim 155, wherein the GALR3 antagonist and the Y5 antagonist are administered repeatedly.

157. A method of claim 155 or claim 156, wherein the
10 galanin receptor antagonist is administered for about 1 week to 2 weeks.

158. A method of claim 155 or claim 156, wherein the Y5 receptor antagonist is administered for about 1 week
15 to 2 weeks.

159. A method of any one of claims 148, 149, 150, or 151, wherein the compound is administered in a pharmaceutical composition comprising a sustained
20 release formulation.

160. A method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a GALR3 receptor
25 agonist effective to decrease nociception in the subject.

161. A method of treating pain in a subject which comprises administering to the subject an amount of
30 a compound which is a GALR3 receptor agonist effective to treat pain in the subject.

162. A method of treating diabetes in a subject which

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comprises administering to the subject an amount of a compound which is a GALR3 receptor antagonist effective to treat diabetes in the subject.

163. A process for determining whether a chemical compound is a GALR3 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the GALR3 receptor with the compound under conditions permitting the activation of the GALR3 receptor, and detecting an increase in GALR3 receptor activity, so as to thereby determine whether the compound is a GALR3 receptor agonist.

164. A process for determining whether a chemical compound is a GALR3 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the GALR3 receptor with the compound in the presence of a known GALR3 receptor agonist, under conditions permitting the activation of the GALR3 receptor, and detecting a decrease in GALR3 receptor activity, so as to thereby determine whether the compound is a GALR3 receptor antagonist.

165. A process of claim 163 or 164, wherein the cells are transfected with nucleic acid encoding, and express GIRK1 and GIRK4.

166. A process of any one of claims 163, 164 or 165, wherein the GALR3 receptor is a mammalian GALR3 receptor.

167. A pharmaceutical composition which comprises an amount of a GALR3 receptor agonist determined by the process of claim 163 effective to increase activity of a GALR3 receptor and a pharmaceutically

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acceptable carrier.

168. A pharmaceutical composition of claim 167, wherein the GALR3 receptor agonist is not previously known.

169. A pharmaceutical composition which comprises an amount of a GALR3 receptor antagonist determined by the process of claim 164 effective to reduce activity of a GALR3 receptor and a pharmaceutically acceptable carrier.

170. A pharmaceutical composition of claim 169, wherein the GALR3 receptor antagonist is not previously known.

171. A process for determining whether a chemical compound specifically binds to and activates a GALR3 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with the chemical compound under conditions suitable for activation of the GALR3 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the GALR3 receptor.

172. The process of claim 171, wherein the second messenger response comprises potassium channel activation and the change in second messenger is an increase in the level of inward potassium current.

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173. A process for determining whether a chemical compound specifically binds to and inhibits activation of a GALR3 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the GALR3 receptor, wherein such cells do not normally express the GALR3 receptor, with both the chemical compound and a second chemical compound known to activate the GALR3 receptor, and with only the second chemical compound, under conditions suitable for activation of the GALR3 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the GALR3 receptor.

174. The process of claim 173, wherein the second messenger response comprises potassium channel activation and the change in second messenger response is a smaller increase in the level of inward potassium current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

175. A process of any one of claims 171, 172, 173 or 174, wherein the GALR3 receptor is a mammalian GALR3 receptor.

176. The process of claim 175, wherein the GALR3 receptor

has substantially the same amino acid sequence as encoded by the plasmid K1086 (ATCC Accession No. 97747).

5   177. The process of claim 175, wherein the GALR3 receptor has substantially the same amino acid sequence as that shown in Figure 2 (Seq. ID No. 2).

178. The process of claim 175, wherein the GALR3 receptor
10   has substantially the same amino acid sequence as encoded by the plasmid pEXJ-hGalR3 (ATCC Accession No. 97827).

179. The process of claim 175, wherein the GALR3 receptor
15   has a sequence, which sequence comprises substantially the same amino acid sequence as that shown in Figure 4 (Seq. ID No. 4) from amino acid 60 through amino acid 427.

20   180. The process of claim 175, wherein the GALR3 receptor has a sequence, which sequence comprises a sequence shown in Figure 4 (Seq. ID No. 4) from amino acid 60 through amino acid 427.

25   181. The process of any one of claims 171, 172, 173, 174, 175, 176, 177, 178, 179 or 180, wherein the cell is an insect cell.

182. The process of any one of claims 171, 172, 173, 174,
30   175, 176, 177, 178, 179 or 180, wherein the cell is a mammalian cell.

183. The process of claim 182, wherein the mammalian cell

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is nonneuronal in origin.

184. The process of claim 183, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

185. The process of claim 184, wherein the nonneuronal cell is the 293 human embryonic kidney cell designated 293-rGALR3-105 (ATCC Accession No. CRL-12287).

186. The process of claim 184, wherein the nonneuronal cell is the LM(tk-) cell designated L-hGALR3-228 (ATCC Accession No. CRL-12373).

187. The process of claim 182, wherein the compound is not previously known to bind to a GALR3 receptor.

188. A compound determined by the process of claim 187.

189. A pharmaceutical composition which comprises an amount of a GALR3 receptor agonist determined by the process of claim 171 or 172 effective to increase activity of a GALR3 receptor and a pharmaceutically acceptable carrier.

190. A pharmaceutical composition of claim 189, wherein the GALR3 receptor agonist is not previously known.

191. A pharmaceutical composition which comprises an amount of a GALR3 receptor antagonist determined by the process of any one of claims 173 or 174

effective to reduce activity of a GALR3 receptor and a pharmaceutically acceptable carrier.

192. A pharmaceutical composition of claim 191, wherein the GALR3 receptor antagonist is not previously known.

193. A method of screening a plurality of chemical compounds not known to activate a GALR3 receptor to identify a compound which activates the GALR3 receptor which comprises:

(a) contacting cells transfected with and expressing the GALR3 receptor with the plurality of compounds not known to activate the GALR3 receptor, under conditions permitting activation of the GALR3 receptor;

(b) determining whether the activity of the GALR3 receptor is increased in the presence of the compounds; and if so

(c) separately determining whether the activation of the GALR3 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the GALR3 receptor.

194. The process of claim 193, wherein the cells are transfected with and expressing GIRK1 and GIRK4.

195. A method of claim 193, wherein the GALR3 receptor is a mammalian GALR3 receptor.

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196. A method of screening a plurality of chemical
compounds not known to inhibit the activation of a
GALR3 receptor to identify a compound which inhibits
the activation of the GALR3 receptor, which
5   comprises:

    (a) contacting cells transfected with and
         expressing the GALR3 receptor with the
         plurality of compounds in the presence of a
10   known GALR3 receptor agonist, under conditions
         permitting activation of the GALR3 receptor;

    (b) determining whether the activation of the GALR3
         receptor is reduced in the presence of the
15   plurality of compounds, relative to the
         activation of the GALR3 receptor in the absence
         of the plurality of compounds; and if so

    (c) separately determining the inhibition of
20   activation of the GALR3 receptor for each
         compound included in the plurality of
         compounds, so as to thereby identify the
         compound which inhibits the activation of the
         GALR3 receptor.

25

197. The process of claim 196, wherein the cells are
     transfected with and expressing GIRK1 and GIRK4.

198. A method of claim 196 or 197, wherein the GALR3
30   receptor is a mammalian GALR3 receptor.

199. A method of any one of claims 193, 194, 195, 196,
     197 or 198, wherein the cell is a mammalian cell.

200. A method of claim 199, wherein the mammalian cell is non-neuronal in origin.

201. The method of claim 200, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

202. A pharmaceutical composition comprising a compound identified by the method of any one of claims 193, 194, or 195 effective to increase GALR3 receptor activity and a pharmaceutically acceptable carrier.

203. A pharmaceutical composition comprising a compound identified by the method of any one of claims 196, 197 or 198 effective to decrease GALR3 receptor activity and a pharmaceutically acceptable carrier.

204. A process of any one of claims 163, 171 or 172, which further comprises determining whether the compound selectively activates the GALR3 receptor relative to another galanin receptor.

205. The process of claim 204, wherein the determination whether the compound selectively activates the GALR3 receptor comprises:

(a) determining the potency of the compound for the GALR3 receptor and for such other galanin receptor; and

(b) comparing the potencies so determined, the presence of a higher potency for the GALR3 receptor than for such other galanin receptor

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indicating that the compound selectively activates the GALR3 receptor.

206. A process of claim 205, wherein such other galanin receptor is a GALR1 receptor.

207. A process of claim 205, wherein such other galanin receptor is a GALR2 receptor.

208. A process of any one of claims 164, 173 or 174, which further comprises determining whether the compound selectively inhibits the activation of the GALR3 receptor relative to another galanin receptor.

209. The process of claim 208, wherein the determination whether the compound selectively inhibits the activation of the GALR3 receptor comprises:

(a) determining the decrease in the potency of a known galanin receptor agonist for the GALR3 receptor in the presence of the compound, relative to the potency of the agonist in the absence of the compound;

(b) determining the decrease in the potency of the agonist for such other galanin receptor in the presence of the compound, relative to the potency of the agonist in the absence of the compound; and

(c) comparing the decrease in potencies so determined, the presence of a greater decrease in potency for the GALR3 receptor than for such

other galanin receptor indicating that the
compound selectively inhibits the activation of
the GALR3 receptor.

5   210. A process of claim 209, wherein such other galanin
receptor is a GALR1 receptor.

211. A process of claim 209, wherein such other galanin
receptor is a GALR2 receptor.

10

212. A process for determining whether a chemical
compound is a GALR3 receptor agonist, which
comprises preparing a cell extract from cells
transfected with and expressing DNA encoding the

15   GALR3 receptor, isolating a membrane fraction from
the cell extract, separately contacting the membrane
fraction with both the chemical compound and GTPγS,
and with only GTPγS, under conditions permitting the
activation of the GALR3 receptor, and detecting

20   GTPγS binding to the membrane fraction, an increase
in GTPγS binding in the presence of the compound
indicating that the chemical compound activates the
GALR3 receptor.

25   213. A process for determining whether a chemical
compound is a GALR3 receptor antagonist, which
comprises preparing a cell extract from cells
transfected with and expressing DNA encoding the
GALR3 receptor, isolating a membrane fraction from

30   the cell extract, separately contacting the membrane
fraction with the chemical compound, GTPγS and a
second chemical compound known to activate the GALR3
receptor, with GTPγS and only the second compound,
and with GTPγS alone, under conditions permitting

35   the activation of the GALR3 receptor, detecting

GTPγS binding to each membrane fraction, and comparing the increase in GTPγS binding in the presence of the compound and the second compound relative to the binding of GTPγS alone, to the increase in GTPγS binding in the presence of the second chemical compound relative to the binding of GTPγS alone, a smaller increase in GTPγS binding in the presence of the compound and the second compound indicating that the compound is a GALR3 receptor antagonist.

214. A process of claim 212 or 213, wherein the GALR3 receptor is a mammalian GALR3 receptor.

215. The process of claim 214, wherein the GALR3 receptor has substantially the same amino acid sequence as encoded by the plasmid K1086 (ATCC Accession No. 97747).

216. The process of claim 214, wherein the GALR3 receptor has substantially the same amino acid sequence as that shown in Figure 2 (Seq. ID No. 2).

217. The process of claim 214, wherein the GALR3 receptor has substantially the same amino acid sequence as encoded by the plasmid pEXJ-hGalR3 (ATCC Accession No. 97827).

218. The process of claim 214, wherein the GALR3 receptor has a sequence, which sequence comprises substantially the same amino acid sequence as that shown in Figure 4 (Seq. ID No. 4) from amino acid 60 through amino acid 427.

219. The process of claim 214, wherein the GALR3 receptor has a sequence, which sequence comprises a sequence shown in Figure 4 (Seq. ID No. 4) from amino acid 60 through amino acid 427.

220. The process of any one of claims 212, 213, 214, 215, 216, 217, 218 or 219, wherein the cell is an insect cell.

221. The process of any one of claims 212, 213, 214, 215, 216, 217, 218 or 219, wherein the cell is a mammalian cell.

222. The process of claim 221, wherein the mammalian cell is nonneuronal in origin.

223. The process of claim 222, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

224. The process of claim 223, wherein the nonneuronal cell is the 293 human embryonic kidney cell designated 293-rGALR3-105 (ATCC Accession No. CRL-12287).

225. The process of claim 223, wherein the nonneuronal cell is the LM(tk-) cell designated L-hGALR3-228 (ATCC Accession No. CRL-12373).

226. The process of claim 221, wherein the compound is not previously known to bind to a GALR3 receptor.

227. A compound determined by the process of claim 226.

FIGURE 1

```
   1 AGCTCCAGCCTAGGCGTTCTACCTGGAAGAATGCAGGGGGCCCAGTACCTAGGACTGAGGA  60
  61 AGATGGCTGACATCCAGAACACATTTCGCTGGACAGCCCAGGGAGCGTAGGGGCTGTGGCAG  120
 121 TGCCTGTGATCTTTGCCCTCATCTTCCTGTTGGGCATGGTGGGCAATGGGCTGGTGTTGG  180
 181 CTGTGCTACTGCAGCCTGCCCAAGTGCCTGGCAGGAGCCAAGCAGTACCACAGATCTCT  240
 241 TCATCCTCAACTTGGCCGTGGCCGACCTTTGCTTCATCCTGTGCCGTGCCCTTCCAGG  300
 301 CAGCCATCTACACACTGGATGCCTGGCTCTTTGGGGCTTCGTGTGCAAGACGGTACATC  360
 361 TGCTCATCTACCTCACCATGTATGCCAGCTTCACCCTGGCCGTCTCCCTGGACA  420
 421 GGTACCTGCTGTGCGCGCCACCCACTGCGCTCCAGAGCCCTGCGCCACCCGCAACGCGC  480
 481 GCGCCGCCGTGGGGGCTCGTGTGGCTGCTCTCTTTCCGCGCCCTACCTAAGCT  540
 541 ATTACGGCACGGTGCGCTACGGCGCTCGAGCTGGGGGCCCGCTTGGGAGGACGCGC  600
 601 GGCGGCCGCGCGCTGGACGTGGCCCACCTTCGCGCGGGCTACCGGTGGCCGTGG  660
 661 TGAGCCTGGCCTACGGACGCACGCTATGTTCCTATGGGCGCCGGGGGTCCGCGGGGCG  720
 721 CGGCGGCAGCAGAGCGCGCAGACGGGCCCGCCGCCACGCGCTCATCCTCTGCTTCTGGT  780
 781 TGGCCGGCTCTACGCGCTTTGCTGGGGGCCCTTCAGCCCGGCCACCTACGCCCCGCCTCG  840
 841 ACGGCCGCTTCGCCCTTCGCCCTTAACCCGTCCTCGCTCTACTCGCTCGCGCCACTTCCGCG  900
 901 CCTACGCCAACTCCTGCCTTAACCCGCTGCCCTGTGGGCCCCTGCGCCGCCACCACCGCGCTC  960
 961 CGCGCTTCCGCCCCTCCGTCGTCGTCTCCAGCCGCGTCTTCGGCCCGCCGGTTATCCGGCGACG  1020
1021 ATCGAGCCCCTGGTTGGAGTGAGCCCAGAGGGATGCTCGTGGTGGGTGGAGAGA  1080
1081 CCAGGCCTCGTGGTTGGAGTATGGCAGACCCTGCCCCGTGTCCCCGCTTGGACTCTGACGTC  1140
1141 CTAGAACTAAACCCTGTCCCCAGGGACCTCAATAACCCTGCCCGCTTGACTCTGACGTC  1200
1201 TGTCAGAATGCCACCAAGAACATCTAGGGAACGGCAGTCTCGCCAGCTCCACCAAAAA  1260
1261 GCAGAAGCAAAGTTGCAGGG  1280
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FIGURE 2

Top column positions: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 370

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V A F A L R R Y R V A V Y A A H A T
A L L Q H D A S A V G A W L R A D E
V V D F V L N L D A A L F C F R G G
A L T P T S R Y E V P M L H H P G G
G G T V K V P P W P G A L S R H Y G
V N S C C A T A A L V R I A S H G R
S G S C V A R S P L A G L L A R A L
G V P L F L L F V Y A A R L H P A
P M E I A T A L C G W R H C S R G D
S G Q F G F R A L A L G H A Y R S G
D L W C F S S A E A F T P Y V R S R Q
L L A L L S R L L F C A G T L G A P P
S F S D W A L L A T L R W A P C P E G
I H P A A Y P W G A T R C P N P Q M R
N L G V D M H V Y V R R L S L W V S P
Q A P A L T R L R D G A A F C L R W S
I F Q L T L V G V L Y E Y A S R R G L
D I L N Y Y A V T A A L F N R L R T
A V L L H L L A G R L A A R A F A P L
M P V I A L Y A Y R S A A G Y R R R R
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Bottom column positions: 1, 21, 41, 61, 81, 101, 121, 141, 161, 181, 201, 221, 241, 261, 281, 301, 321, 341, 361

FIGURE 3

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   1  CACTCAGGCGATGACTTTGGCTCTGCTCTCCCTCCCATCTCCCACGAGCTTCCAGCCC      60
  61  AGAACACCTGGCCAGACCCAGGTCGGGGGAGTTAGATCCCGGGGTCAAGCAACCAGAACT    120
 121  GGGGGCTCTTGCCTGAGGATTCCAGCTTCTCTTCCCAGGTGCCCGTCTGATGGGGAGATG    180
 181  GCTGATGCCCAGAACATTTCACTGGACAGCCCAGGAGTGTGGGGGCCGTGGCAGTGCCT     240
 241  GTGGTCTTTGCCCTAATCTTCCTGCTGGGCACAGTGGGCAATGGGCTGGTGGCTGGCAGTG   300
 301  CTCCTGCAGCCTGGCCCGAGTGCCTGGCAGGAGCCTGGCAGCACGGGACCTGTTCATC     360
 361  CTCAACCTGGCGGTGGCTGACCTTCTGCTTCATCCTGTGCTGCCCTTCCAGGCCACC      420
 421  ATCTACACGCTGGATGCCTGGCTCTTTGGGGCCCTCGTCTGCAAGGCCGTGCACCTGCTC    480
 481  ATCTACCTCACCATGTACGCCAGCAGCTTTACGCTTCTGTGCTGCTTCCGTGGACAGGTAC   540
 541  CTGGCCGTGCGGCCACCCGGCTGCGCCCCTCCGCGCGGCCCCTAACGCCCCGCGCCC      600
 601  GCAGTGGGCGTGGGCGTGGCTGCTCTGGCTGGAGCTCTGCCGCTGCCCGTGGTGAGC      660
 661  GGCACCGTGCGCTACGGCCGCGCTACGGGGACGCGGCCCGC                      720
 721  CGCGCCCTGGACGTGGCCACCTTCGCGCTGCCCGGCTACCTGCCGTGGTGCCCGTGGGCCGG   780
 781  CTGGCCTACGGGCCCGGAGGGCGCACGCTGCCTGCTTCCTGGCGCTTCCTGTGGCCCGCGGCG   840
 841  GCGGGCCGAGGGCCGGCGCTGGGCGCGGCCCGGCCACCGGCCCGCGGCGGGTGGCC       900
 901  GCGCTCTACGGGCGCTCTGCTGGGGTCCGCACCTACGCGCCTGCTTCATCCTGCGTTTCTGT TGCTTGGCGGC   960
 961  CGCTTCGCCCTTCAGCCCGGCCTTCGCGTCCGCCTACGCGCCTGCCTCACTGCCCTGCCTAC  1020
1021  GCCAACTCCTGCCTCAACCCCGTGCCGTGCCGGCCCGACGCGCCACCGTGCCGGCCTTGCGT  1080
1081  TTCCGCCGCCGTGTGCCGTGGCCGTCCTCGGGGCCCACCGGGCCCGGAGAGCGCCCGGCCTAGCGCGGG 1140
1141  CGGGTCCCCGCGCTCCGGGGGCTGCTGGTGGTCCCGAGGCGCCCAGGAGGACCCGTCCACGGCGGA    1200
1201  AGGCTGGCTGGTCCCGAGGACCGGAATAAACCCTGCCGCCTGGACTCCGCCTGTCCGTGTC  1260
1261  GAGGCTGCCCGAGGACCGGAATAAACCCTGCCGCCACCGGGCCCAGGGATGGGCCAATGCCACGAGC  1320
1321  TCACTCCCGTTCTCCGAAGGCCGGCACGCGCCTGCTGTGTCCGTGTC  1380
1381  TCTCTGAGGGGCCGTTGAGTGGGAGCCGACTTGTCCCCGC                        1417
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FIGURE 4

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        20  40  60  80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440

    P   T   M   P   V   I   T   L   Y   A   Y   R   S   A   A   G   Y   R   R   G   G
    Q   R   E   V   A   F   A   L   R   R   Y   R   V   A   V   Y   A   A   L   S   G   G
    F   T   G   A   L   L   Q   H   D   A   S   A   V   G   A   W   L   R   A   P   H
    S   A   D   V   V   D   F   V   V   N   L   D   A   A   L   F   C   H   F   R   R   V
    T   Q   S   A   L   T   P   A   S   R   Y   E   V   P   M   C   H   H   R   A   P
    P   G   P   G   G   T   V   K   V   P   P   W   P   G   A   L   S   R   A   D   G
    S   R   C   V   N   S   C   C   A   T   A   A   L   V   R   I   A   S   R   G   E
    P   S   R   S   G   G   C   V   A   R   S   P   L   A   G   L   L   A   H   P   R
    P   P   R   P   G   V   P   L   L   L   F   V   Y   A   A   R   L   R   C   P
    P   V   L   P   T   E   H   A   T   A   L   C   G   W   R   H   C   A   R   G   E
    S   G   S   S   G   Q   F   G   F   R   A   L   A   L   G   H   A   Y   R   P   P
    L   G   A   D   L   W   C   F   S   A   E   A   F   T   P   Y   V   R   P   G
    L   V   P   L   L   A   L   L   S   R   L   L   F   R   A   G   T   L   G   G   Q
    A   Q   I   S   F   S   D   W   A   L   L   A   T   L   R   W   A   P   C   S   G   E
    L   T   R   H   H   P   A   A   Y   P   W   G   A   T   R   C   P   N   P   S   G   P
    T   Q   L   N   L   G   V   D   M   H   V   Y   V   R   R   L   S   L   W   A   G   G
    M   G   C   Q   A   P   A   L   T   R   L   R   D   G   A   A   F   C   L   P   A   R
    A   P   S   A   F   Q   L   T   L   V   G   V   L   Y   E   Y   A   S   R   R   L   A
    S   T   G   D   V   L   N   Y   Y   A   V   T   A   A   A   L   F   N   R   V   L   A
    H   R   G   A   V   L   L   H   I   L   A   G   R   L   A   A   R   A   F   R   R   E

     1  21  41  61  81 101 121 141 161 181 201 221 241 261 281 301 321 341 361 381 401 421
```

FIGURE 5A

```
hGALR3   . . . . . . M A D A Q N I S L D S P G . . . .       13
rGALR3   . . . . . . M A D I Q N I S L D S P G . . . .       13
rGALR1   M E L A P V N L S E G N G S D P E P P A E P R P L   25

                     I
hGALR3   . . . . S V G A V A V P V V F A L I F L L G T V G N 35
rGALR3   . . . . S V G A V A V P V I F A L I F L L G M V G N 35
rGALR1   F G I G V E N F I T L V V F G L I F A M G V L G N   50

hGALR3   G L V L A V L L Q P G P S A W Q E P G S T T D L F   60
rGALR3   G L V L A V L L Q P G P S A W Q E P S S T T D L F   60
rGALR1   S L V I T V L A R S K P G . K P R S T T N L F       72

         II
hGALR3   I L N L A V A D L C F I L C C V P F Q A T I Y T L   85
rGALR3   I L N L A V A D L C F I L C C V P F Q A A I Y T L   85
rGALR1   I L N L S I A D L A Y L L F C I P F Q A T V Y A L   97
```

FIGURE 5B

```
                                                      III
hGALR3  D A W L F G A L V C K A V H L L I Y L T M Y A S S   110
rGALR3  D A W L F G A F V C K T V H L L I Y L T M Y A S S   110
rGALR1  P T W V L G A F I C K F I H Y F F T V S M L V S I   122

hGALR3  F T L A A V S V D R Y L A V R H P L R S R A L R T   135
rGALR3  F T L A A V S L D R Y L A V R H P L R S R A L R T   135
rGALR1  F T L A A M S V D R Y V A I V H S R R S S L R V     147

                  IV
hGALR3  P R N A R A A V G L V W L L A A L F S A P Y L S Y   160
rGALR3  P R N A R A A V G L V W L L A A L F S A P Y L S Y   160
rGALR1  S R N A L L G V G F I W A L S I A M A S P . V A Y   171

hGALR3  Y G T V . . R Y G A L E L C V P A W . E D A R R R   182
rGALR3  Y G T V . . R Y G A L E L C V P A W . E D A R R R   182
rGALR1  Y Q R L F H R D S N Q T F C W E H W P N Q L H K K   196
```

FIGURE 5C

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                        V
hGALR3  A L D V A T F A A G Y L L P V A V V S L A Y G R T   207
rGALR3  A L D V A T F A A G Y L L P V A V V S L A Y G R T   207
rGALR1  A Y V V C T F V F G Y L L P L L I C F C Y A K V     221

hGALR3  L R F L W A A V G P A G A A A A E A R R R A T G R   232
rGALR3  L C F L W A A V G P A G A A A A E A R R R A T G R   232
rGALR1  L N H L H K K L K N M S K K S E A S K K · · · K     242

                   VI
hGALR3  A G R A M L A V A A L Y A L C W G P H H A L I L C   257
rGALR3  A G R A M L A V A A L Y A L C W G P H H A L I L C   257
rGALR1  T A Q T V L V V V V F G I S W L P H H V I H L W     267

                        VII
hGALR3  F W Y G R F A F S P A T Y A C R L A S H C L A Y A   282
rGALR3  F W Y G R F A F S P A T Y A C R L A S H C L A Y A   282
rGALR1  A E F G A F P L T P A S F F F R I T A H C L A Y S   292
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FIGURE 5D

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hGALR3  N S C L N P L V Y A L A S R H F R A R F R R L W P   307
rGALR3  N S C L N P L V Y S L A S R H F R A R F R R L W P   307
rGALR1  N S S V N P I I Y A F L S E N F R K A Y K Q V F K   317

hGALR3  C G R R · R H R A R R A L R R V R P A S S G   329
rGALR3  C G R R · R H H H R A H R A L R R V Q P A S S G   332
rGALR1  C R V C N E S P H G D A K E K N R I D T P P S T N   342

hGALR3  P P G C P G D A R P S G R L L A G G G Q G P E P R   354
rGALR3  P A G Y P G D A R P R G W S M · · · · E P R   350
rGALR1  C T H V · · · · · · · · · · · · · ·   346

hGALR3  E G P V H G G E · A A R G P E   368
rGALR3  G D A L R G G G E T R L T L S P R G P Q   370
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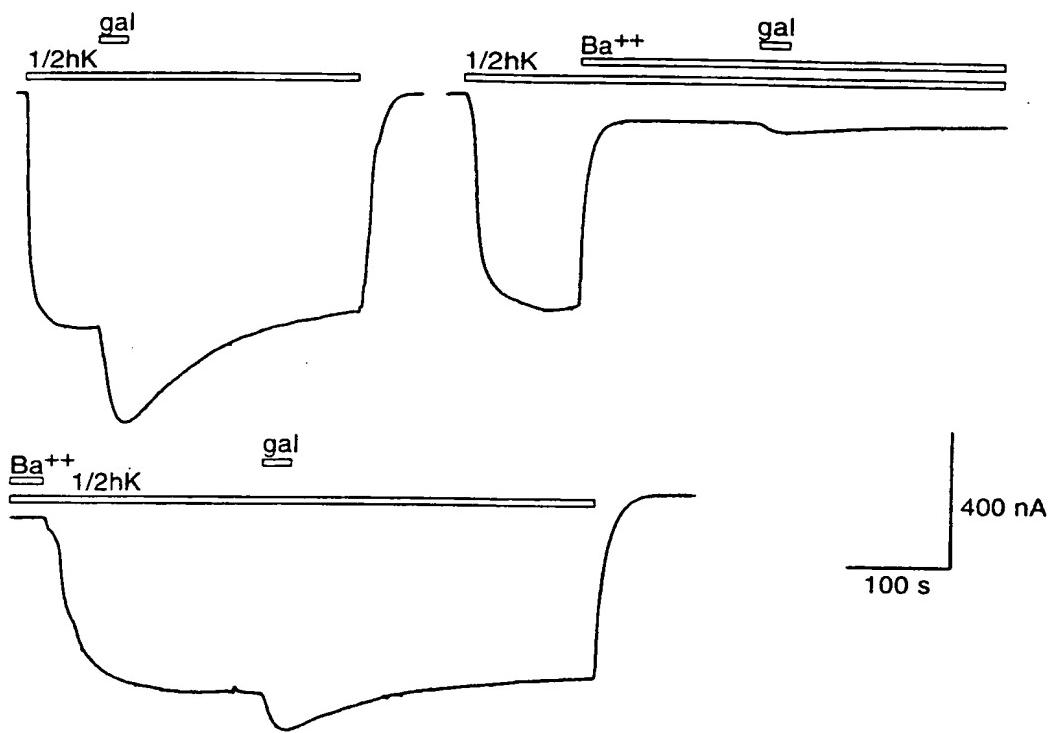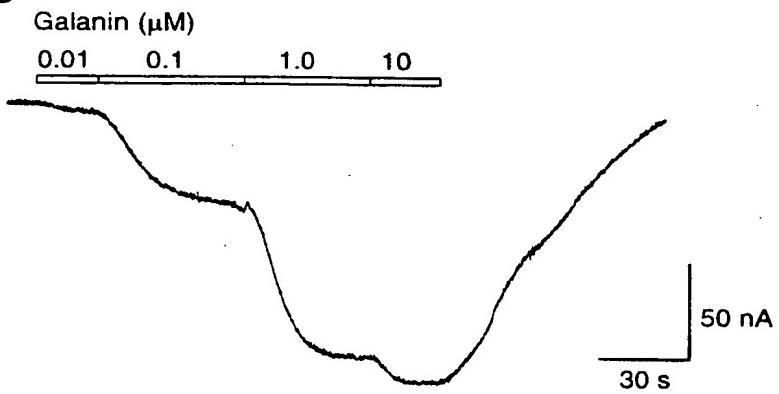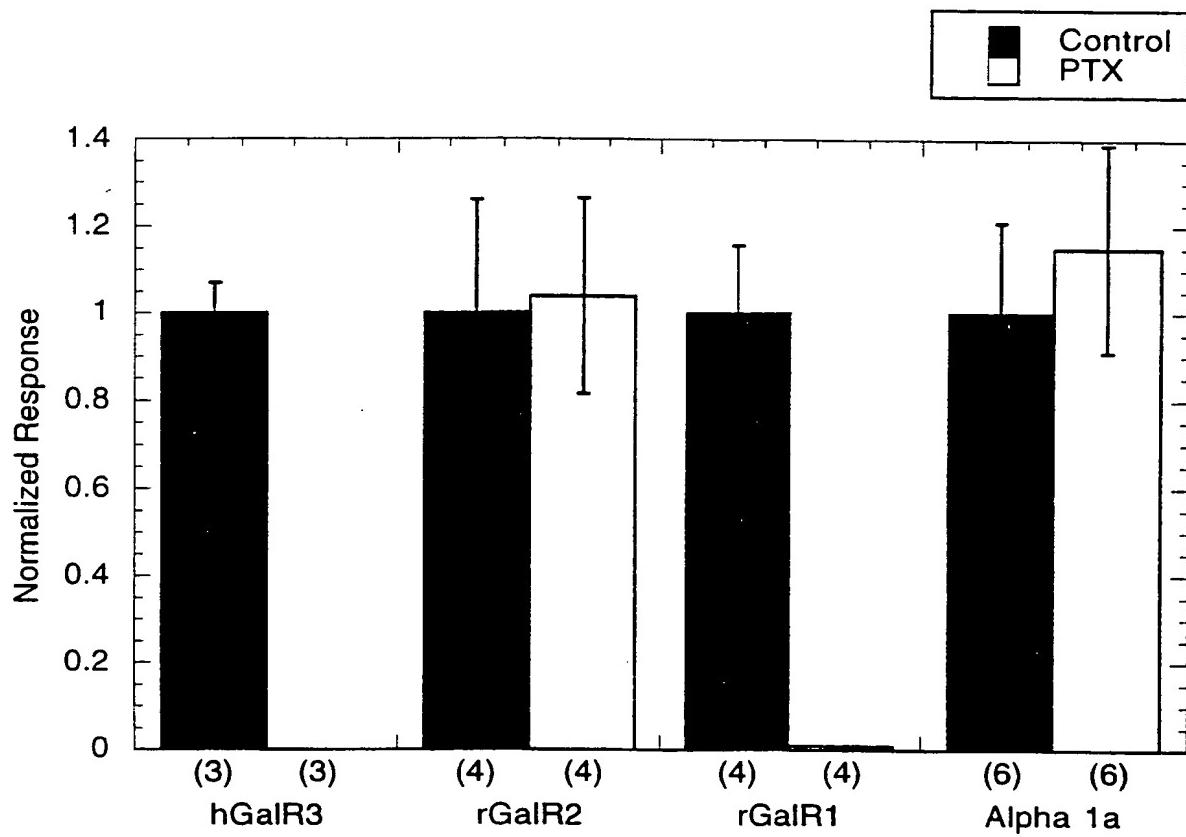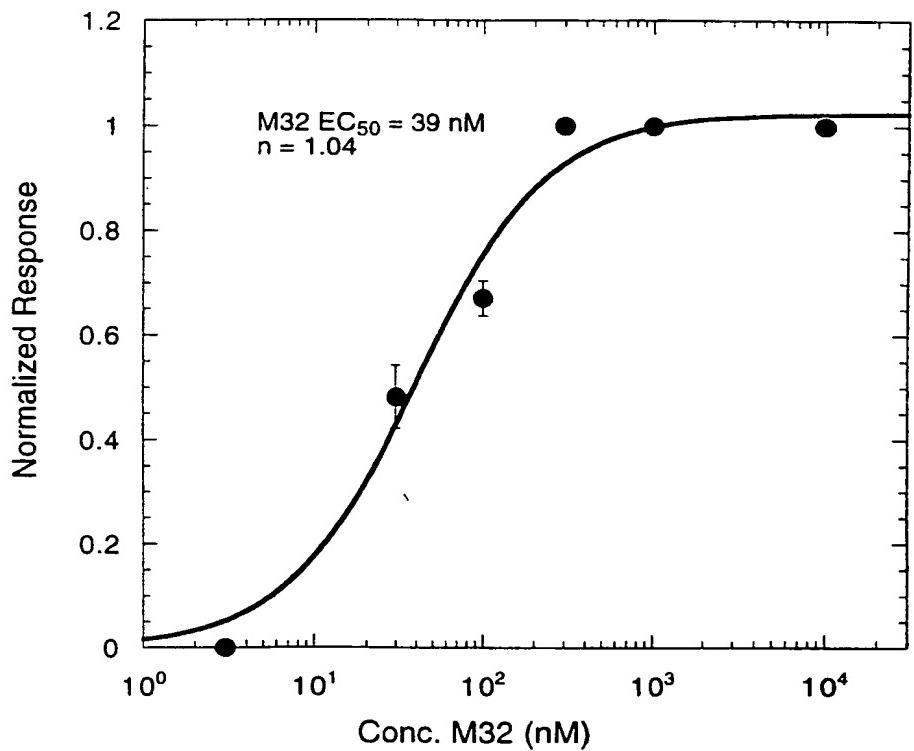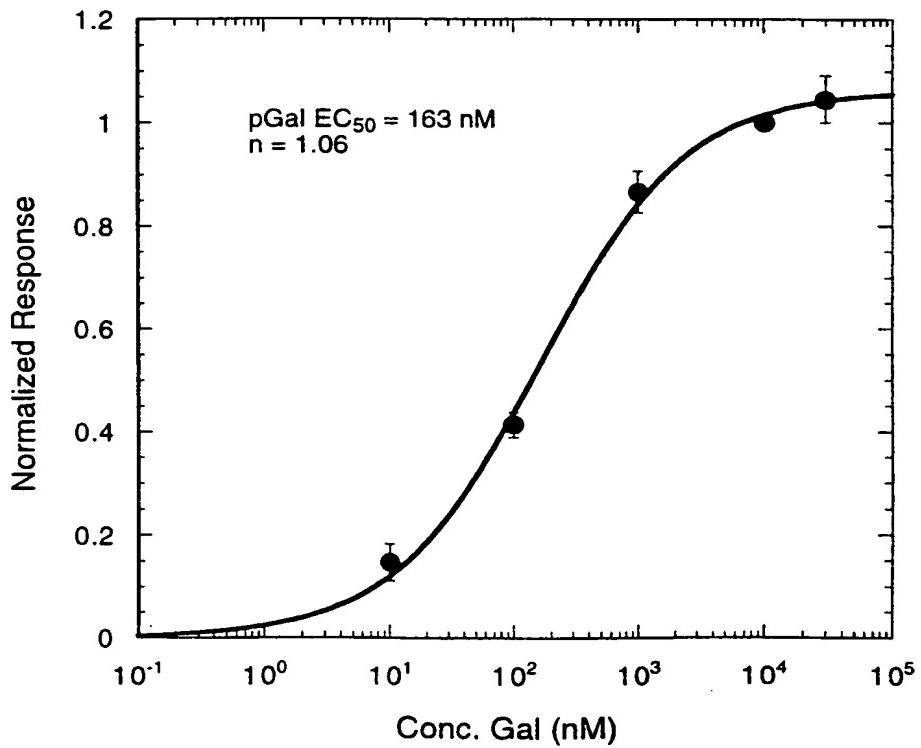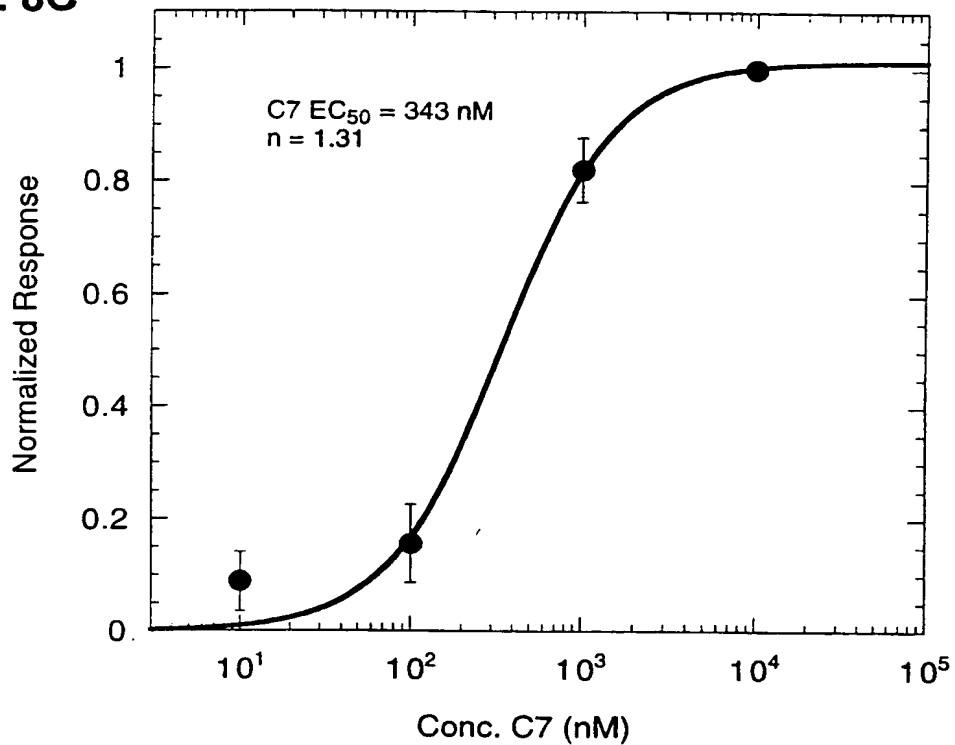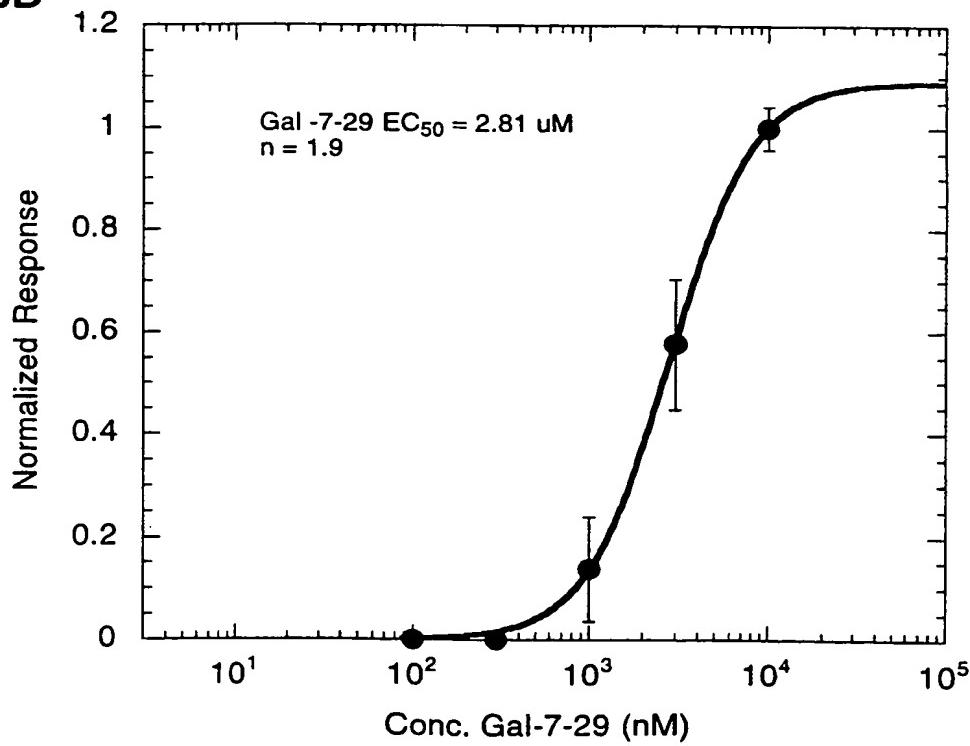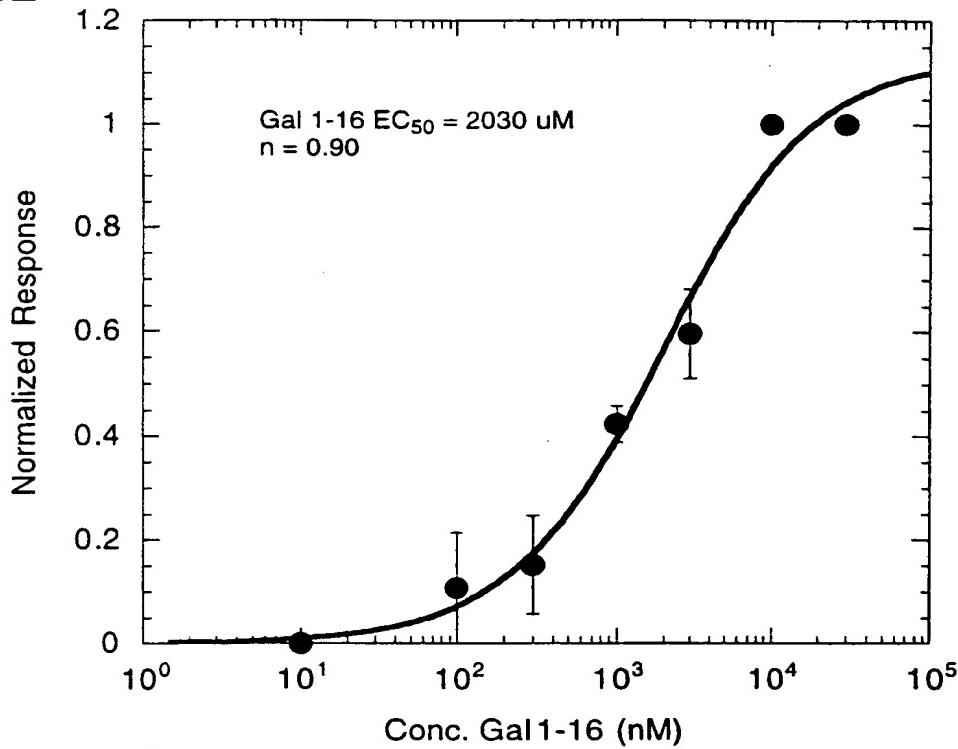
FIG. 6A

FIG. 6B

FIG. 7

FIG. 8A

FIG. 8B

FIG. 8C

C7 EC$_{50}$ = 343 nM
n = 1.31

Normalized Response

Conc. C7 (nM)

FIG. 8D

Gal -7-29 EC$_{50}$ = 2.81 uM
n = 1.9

Normalized Response

Conc. Gal-7-29 (nM)

FIG. 8E

Gal 1-16 EC$_{50}$ = 2030 uM
n = 0.90

X-axis: Conc. Gal 1-16 (nM)
Y-axis: Normalized Response

FIG. 8F

M40 EC$_{50}$ = 2265 nM
n = 0.57

X-axis: Conc. M40 (nM)
Y-axis: Normalized Response

FIG. 8G

●   ●

FIG. 9A

FIG. 9B

FIG. 10
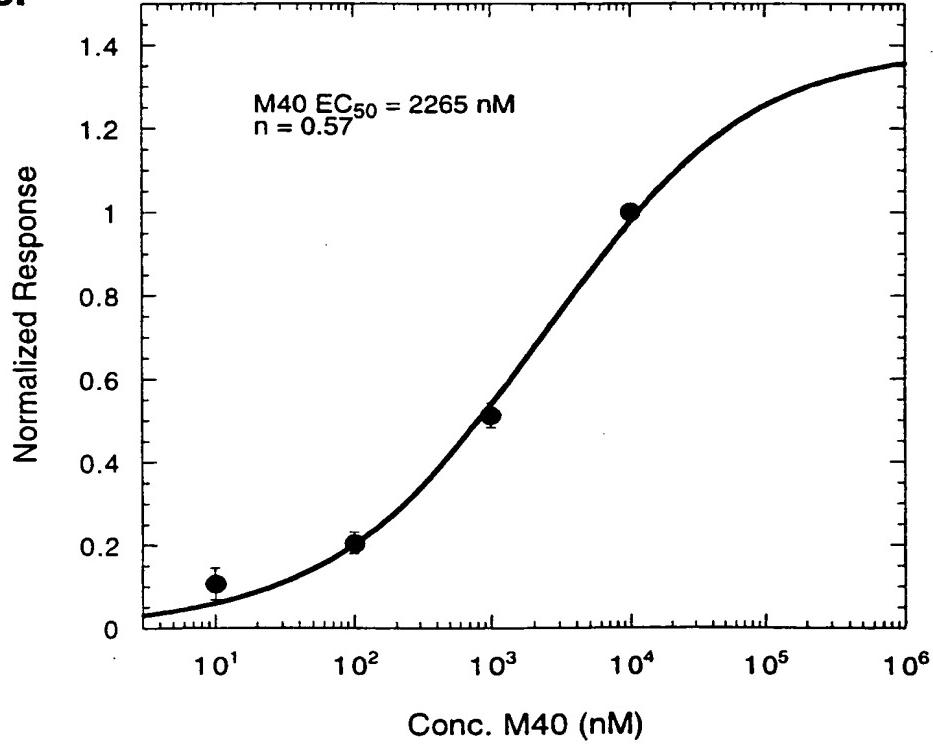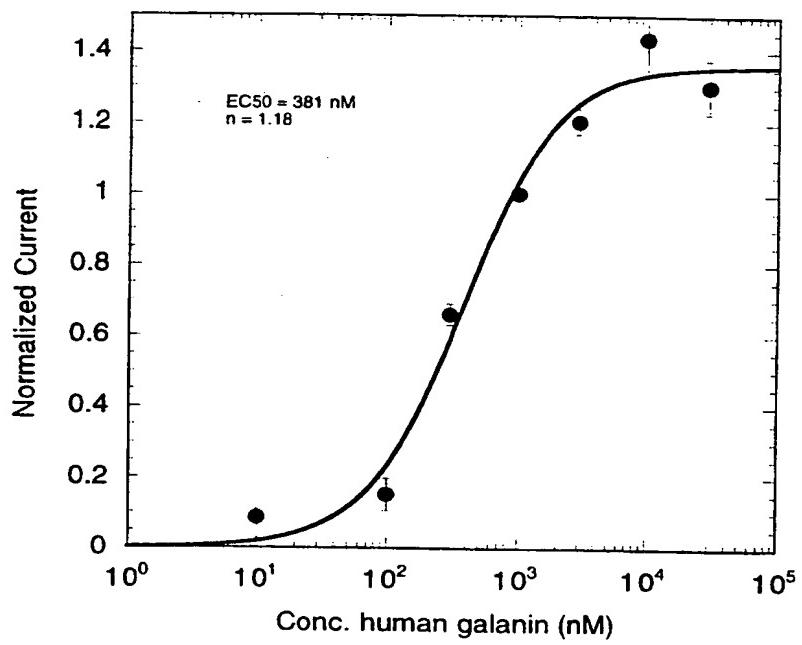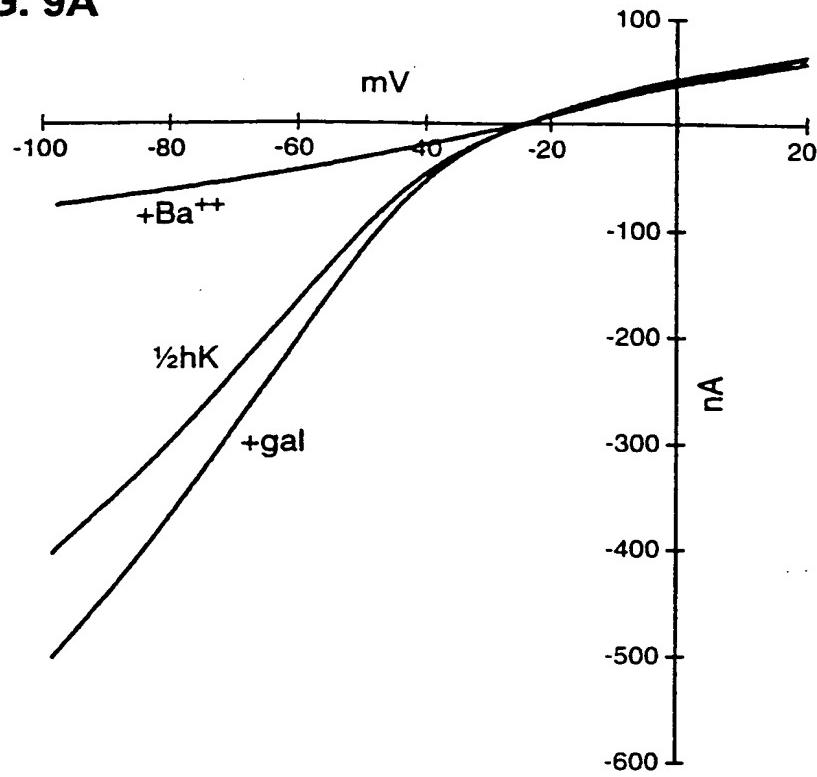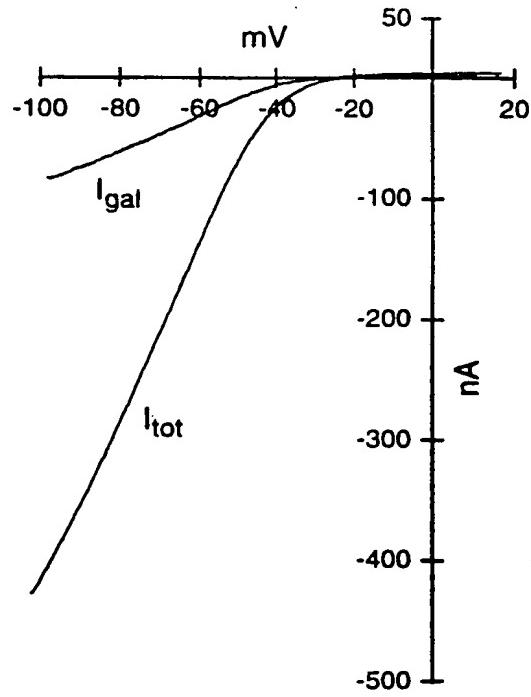
INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/18222

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

    Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1-53, 74-84, 86-90, 92-106, 108-113, 128-129, 144-147

Remark on Protest
☐ . The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07K 1/14, 14/705; C12N 5/10, 15/12, 15/63; C12P 21/00; G01N 33/53, 33/566

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

- 435/7.2, 29, 69.1, 320.1, 325, 348, 354, 356, 357, 358, 365, 369; 530/350, 412; 536/23.5, 24.3, 24.5; 514/44

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-53, 74-84, 86-90, 92-106, 108-113, 128-129, 144-147, drawn to an isolated nucleic acid, a GALR3 receptor, a vector, a cell, a membrane preparation, a nucleic acid probe encoding the GALR3 receptor, a process for identifying a chemical compound which binds GALR3, a process involving competitive binding, a method for screening a plurality of chemical compounds, a method of preparing the GALR3 receptor, a process for identifying a chemical compound which binds GALR3 and selectively binds GALR3 relative to other galanin receptor, and a process of determining whether a compound binds GALR3 and another galanin receptor.

Group II, claim(s) 54-56 and 62-66, drawn to an antisense oligonucleotide and a pharmaceutical composition comprising antisense oligonucleotide.

Group III, claim(s) 57-61, and 67, drawn to an antibody and a pharmaceutical composition comprising the antibody.

Group IV, claim(s)68 and 71-73, drawn to a transgenic nonhuman mammal expressing DNA encoding a GALR3 receptor.

Group V, claim(s)69 and 71-73, drawn to a transgenic nonhuman mammal comprising a homologous recombination knockout of the native GALR3 receptor.

Group VI, claim(s) 70 and 73, drawn to a transgenic nonhuman mammal comprising antisense DNA complementary to DNA encoding a GALR3 receptor.

Group VII, claim(s) 85, 91, 107, 119-120, 123-124, 167-170, 188-192 and 227, drawn to drawn to a compound that bind GALR3, an antagonist identified by administration into transgenic nonhuman mammal and pharmaceutical composition comprising the antagonist, a pharmaceutical composition comprising a GALR3 antagonist, an agonist identified by administration into transgenic nonhuman mammal and pharmaceutical composition comprising the agonist, a pharmaceutical composition comprising a GALR3 agonist, a compound identified by activation of GALR3, a pharmaceutical composition comprising an agonist, and pharmaceutical composition comprising an antagonist.

Group VIII, claim(s) 114, drawn to a method of detecting expression of a GALR3 receptor by detecting the presence of mRNA.

Group IX, claim(s) 115, drawn to a method of detecting GALR3 with the antibody.

Group X, claim(s) 116-117, drawn to a method of determining the physiological effects of GALR3 receptor in a transgenic non-human mammal.

Group XI, claim(s) 118 and 122, drawn to a method for identifying an antagonist by administration into transgenic nonhuman mammal, and a method for identifying an agonist by administration into transgenic nonhuman mammal.

Group XII, claim(s) 121, 130-143, 148-159, and 162, drawn to a method of treating an abnormality in a subject comprising administering an antagonist, a method of modifying feeding behavior by administering an antagonist, a method of decreasing feeding behavior by administering GALR3 antagonist and Y5 receptor antagonist, a method of treating diabetes by administering an antagonist.

Form PCT/ISA/210 (extra sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/18222

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   435/7.2, 29, 69.1, 320.1, 325, 348, 354, 356, 357, 358, 365, 369; 530/350, 412; 536/23.5, 24.3, 24.5; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN, BIOSCIENCE, CAPLUS, BIOSIS, MEDLINE, EMBASE, SCISEARCH

search terms: galanin?(5a)receptor?, galanin?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Please See Continuation of Second Sheet.	

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 DECEMBER 1997	03 FEB 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer MICHAEL D. PAK
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y ----- A	PARKER et al. Cloning and characterization of the rat GAIR1 galanin receptor from Rin 14B insulinoma cells. Molecular Brain Research. 28 December 1995, vol. 34, No.2, pages 179-189, especially pages 181-184.	1-3, 5-9, 11, 13, 15, 17, 22-25, 30, 31, 35-36, 38, 39, 45, 74-78, 80, 81, 87-89, 93-96, 98, 99 -------- 19-21, 26-29, 37, 42-44, 84, 86, 90, 92, 108-113, 128-129, 144-147 ---------- 4, 10, 12, 14, 16, 18, 32-34, 40, 41, 46-53, 79, 82-83, 97, 100-106
X ------ Y ------- A	WO 95/22608 A1 (RHONE-POULENC RORER S.A.) 24 August 1995 (24/08/95), see especially pages 2-4, 7, and 15-19.	1-7, 9, 11, 13, 15, 17, 22, 23-25, 27, 30-31, 35-36, 38, 39, 45, 74-78, 80-81, 87-89, 93-96, 98, 99, 109-113, 128-129 ---------- 19-21, 26, 28-29, 37, 42-44, 84, 86, 90, 92, 108, 144-147 ----------- 8, 10, 12, 14, 16, 18, 32-34, 40-41, 46-53, 80, 82-83, 97, 100-106
Y	BOUVIER, M. et al. Dynamic palmitoylation of G-protein-coupled receptors in eukaryotic cells. Methods in Enzymology. 1995, Vol. 250, pages 300-314, especially pages 304-308.	28-29, 42-44, 86
Y	US 5,462,856 A (LERNER et al.) 31 October 1995 (31/10/95), see especially columns 1-5.	26, 37

Group XIII, claim(s) 125, 130-138, and 160-161, drawn to a method of treating an abnormality in a subject comprising administering an agonist, a method for modifying the feeding behavior by administering an agonist, a method of decreasing nociception by administering an agonist, a method of treating pain by administering an agonist.

Group XIV, claim(s) 126-127, drawn to a method for diagnosing a predisposition to a disorder.

Group XV, claim(s) 163-166, 171-187, and 193-226, drawn to a process of determining whether a chemical compound is a GALR3 receptor agonist comprising activation of the GALR3 receptor, and a process of determining whether a chemical compound is a GALR3 receptor antagonist comprising inhibition of the activation of the GALR3 receptor, a process for determining a chemical compounds activates GALR3 receptor, a process for determining a chemical compounds activates GALR3 receptor, a method for screening a plurality of chemical compound by activation of GALR3, a method for screening a plurality of chemical compound which inhibits activation of GALR3, a process for determining whether a chemical compound is a GALR3 agonist comprising the activation of GALR3 with GTP and a chemical compound, and a process for determining whether a chemical compound is a GALR3 antagonist comprising the activation of GALR3 with GTP and a chemical compound.

and it considers that the International Application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The inventions listed as Groups I-XV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The main invention is Group I which is first product, first method of making the product, and the first method of using the product. Pursuant to 37 CFR 1.475(d), these claims are considered by the ISA/US to constitute the main invention, and none of the related groups II-XV correspond to the main invention.

The products of Group II-VII, do not share the same or corresponding special technical feature with Group I, because they are drawn to products having materially different structures and functions, and each defines a separate invention over the art.

The methods of Groups VIII-XV, do not share the same or corresponding special technical feature with Group I, because the methods have materially different process steps and are practiced for materially different purposes, and each defines a separate invention over the art.

Therefore, the claims are no so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

Form PCT/ISA/210 (extra sheet)(July 1992)*

PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : C07K 1/14, 14/705, C12N 5/10, 15/12, 15/63, C12P 21/00, G01N 33/53, 33/566	A1	(11) International Publication Number: **WO 98/15570**
		(43) International Publication Date: 16 April 1998 (16.04.98)

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(74) Agent: WHITE, John, P.; Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY 10036 (US).

(54) Title: DNA ENCODING GALANIN GALR3 RECEPTORS AND USES THEREOF

(57) Abstract

This invention provides an isolated nucleic acid encoding a mammalian galanin receptor, an isolated galanin receptor protein, vectors comprising isolated nucleic acid encoding a mammalian galanin receptor, cells comprising such vectors, antibodies directed to a mammalian galanin receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian galanin receptor, antisense oligonucleotides complementary to unique sequences of nucleic acid encoding a mammalian galanin receptor, nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian galanin receptor, as well as methods of determining binding of compounds to mammalian galanin receptors.